BACTERIOLOGY

FOR MEDICAL STUDENTS AND PRACTITIONERS

OXFORD UNIVERSITY PRESS
AMEN HOUSE, E.C. 4
London Edinburgh Glasgow New York
Toronto Melbourne Capetown Bombay
Calcutta Madras
HUMPHREY MILFORD
PUBLISHER TO THE UNIVERSITY

OXFORD MEDICAL PUBLICATIONS

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SECOND EDITION

OXFORD UNIVERSITY PRESS
LONDON : HUMPHREY MILFORD
1938

First Edition 1933
Second Edition 1938

PRINTED IN GREAT BRITAIN

PREFACE TO THE FIRST EDITION

THE chief aim of this book is to present shortly, readably, and relevantly as much of the vast subject of bacteriology as a medical student or practitioner needs to know; leaving details of technique to a practical course, and emphasizing the wider biological relations of microbe and man.

This method of presentation may, if successful, help to correct the feeling, surprisingly common among students, that bacteriology is a dull subject. An impression so mistaken as this can only arise from faulty presentation; from the attempt to inculcate too many facts, and the failure to make them sufficiently interesting and relevant to the student's purpose in life.

I wish to acknowledge most gratefully the help of various colleagues and friends: Dr. R. L. Vollum, who has corrected for me many errors and obscurities; Professor G. Dreyer and Dr. E. W. Ainley Walker, who have given me encouragement and many valuable suggestions; and finally Mr. D. H. Waterfield, whose literary judgement has helped me to eliminate many faults of expression.

A. D. G.

Sept., 1933.

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CHAPTER I

BACTERIA AND THEIR SIGNIFICANCE TO MAN

Discovery of Microbes. The Germ Theory of Disease

In 1675 Leeuwenhoek, a linen-draper of Delft, turning his improved microscope to the examination of stagnant water, saw minute rod-shaped or spherical organisms which he describes as 'one thousand times smaller than the eye of a big louse'. Soon after this he observed similar objects in scrapings from his teeth, and his drawings leave no doubt that they were bacteria. He had, however, no inkling of their significance, and two hundred years had to pass before it was realized.

Since the earliest times chemists had regarded the fermentation of sugary fluids as an interesting and obscure phenomenon, and had considered as a chemical substance the slimy matter (yeast) that collects on the bottom of the vessel. This belief persisted until the middle of the nineteenth century, when yeast was shown to be a microscopic plant or fungus.

The truth of this discovery, however, was not generally accepted until Louis Pasteur, the brilliant French chemist, proved in 1854 that in the fermentation of sugar and other substances the production of alcohol, CO_2 gas, and organic acids is due to the vital activity of yeasts and bacteria.

This glimpse of microbes as the cause of chemical degradation of organic matter led Pasteur to investigate the 'diseases' of wine and beer, which he soon showed to be due to the growth of fungi, and to be preventible by the exclusion of these contaminating

organisms, or by heat-sterilization of the finished and bottled product.

The 'diseases of wine and beer' having been proved microbial, why not also those of animals and man? An epidemic disease of silkworms was disorganizing the silk industry in France; Pasteur at once started investigations, and although he did not identify the causative microbe (a parasitic protozoon), he showed that the disease could be eradicated by acting on the assumption that a microbe was at work. From this he passed to diseases of animals and man, and made a series of discoveries of the first importance which went far to establish the Germ Theory of disease. His experimental proof that microbes are not generated spontaneously out of dead organic matter is one of the classics of scientific research.

Hardly second to Pasteur in importance was Robert Koch, who, working independently in Germany, proved in 1876 that *Bacillus anthracis* is the cause of anthrax in animals and man; and that blood-poisoning (septicaemia) is due to the invasion of the body by bacteria. Most of the technical methods which we practise to-day were invented by these two men.

Meanwhile Joseph Lister of Edinburgh had long been searching for the causes of wound-putrefaction, when Pasteur's publications on fermentation showed him the tremendous possibilities of the Germ Theory. If it is the entry of microbes into wounds during operations that sets up suppuration or gangrene, steps should clearly be taken to prevent it. By installing a carbolic spray in the operating-theatre, to kill all bacteria that might drop into the wound, Lister effected an immediate and astonishing reduction of operative wound-sepsis. Nevertheless, it took a long time for the method to gain acceptance among surgeons. The 'aseptic' (germ-excluding) technique, to

which the advance of modern surgery has been chiefly due, is the direct development of Lister's 'antiseptic' (germ-destroying) method.

General Characters and Activities of Bacteria

Bacteria are minute unicellular plants of very simple structure, devoid of chlorophyll and showing no conjugation or other sign of sexual life. The individual cells are so small that aggregates of 1,000 or more are only just perceptible by the naked eye. Under the microscope, with a magnification of 500 to 1,000 diameters, they appear in one or other of the following forms:

Form	*Name*
Spheroid	Coccus
Straight Rod	Bacterium, Bacillus, and various other names
Curved Rod	Vibrio
Spiral Rod	Spirillum and Spirochaete
Branching threads (filaments)	Actinomyces

The botanical name for bacteria is 'Schizomycetes', i.e. splitting fungi. It indicates that they have much in common with the true fungi, but that they reproduce solely by the fission of a full-grown cell into two halves. Bacteria may also properly be called 'microbes', a term however, which includes other microscopic organisms, such as yeasts, moulds, and pathogenic (disease-causing) protozoa.

The Distribution, Life-habits, and Significance of Bacteria

The soil is teeming with bacteria; the purest natural water is not free from them. In Arctic snow and in the

hot springs of America they survive and even multiply. They are very numerous in the dusty air of towns and houses, but scanty in the pure atmosphere of mountains and open plains.

If we expose a flat dish of meat-infusion-jelly for

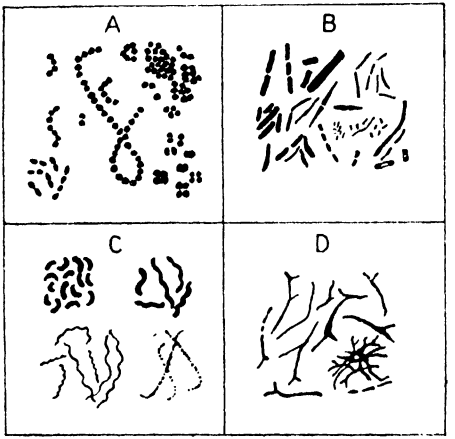

FIG. 1. Forms of Bacteria

A. *Cocci*. B. Rods: *Bacterium*, *Bacillus*, &c. C. Spiral organisms (*Vibrio*, *Spirillum*, *Spirochaetes*). D. *Actinomyces*.

a few minutes in any inhabited room, then cover it and put it in a dark, warm cupboard over-night, we shall see in the morning little white or coloured disks on the surface of the jelly. These are 'colonies' of bacteria, formed by the multiplication of single cells, which were deposited out of the air in those few minutes.

Life-habits. (1) *Saprophytes*. The great majority of bacteria are harmless to man and animals. They live on decaying organic matter in soil and water, and are

therefore classed as *saprophytes*. Many of them, however, are important to us as the cause of the souring and putrefaction of foodstuffs, and as fertilizing the soil by their chemical actions.

(2) *Parasites*. A good many species have become adapted to a life on or in the bodies of larger creatures, and the association may take any of three forms; viz. (*a*) *Symbiosis*, (*b*) *Commensalism*, (*c*) *Pathogenicity*. These varieties of parasitism are only distinguishable clearly in their typical forms, since each shades over imperceptibly into the others.

(*a*) *Symbiosis*. Certain plants and insects tolerate the growth of bacteria and moulds in their tissues, and even thrive better with than without them. One of the best-known instances is the growth of a bacterium (*Rhizobium leguminosarum*) in the root-nodules of leguminous plants. This bacterium has the power of 'fixing' atmospheric nitrogen and of synthesizing the nitrites and nitrates which nourish the plant and enrich the soil. Although many other interesting cases of symbiosis are known in plants and insects, none has been yet described in the higher animals or man.

(*b*) *Commensalism*. Certain bacteria live on the external and internal surfaces of the body, nourished by the secretions of the skin or by the half-digested contents of the intestine (Table XXVI, p. 266). Although they are normally harmless, some of them are able on occasions to cause inflammation, if they gain access to the tissues owing to local damage or functional disturbance (e.g. *Bact. coli*, p. 97).

(*c*) *Pathogenicity*. A limited number of species are able to grow and multiply in the tissues, where their body-constituents or excretions irritate and damage the cells. The symptoms of the various infective diseases are the direct or indirect result of this damage.

At the same time it provokes a strong defence-reaction (Chap. XV), which generally succeeds in eliminating the invader. During the course of the illness, however, a few of the bacteria are generally transferred by some means or other to a fresh 'host', so that the species is not exterminated.

Infection and invasion. The transference of pathogenic microbes to a susceptible subject is called *Infection*. Disease, however, does not result unless *invasion* of the tissues takes place. This term must not be taken literally, since bacteria have little or no power of *active* progress through tissues; and of course they are innocent of malicious intention. Invasion really consists of a passive distribution of the bacteria by the movements of blood or lymph, and by wandering phagocytes which have ingested but failed to digest the bacteria (p. 230).

Vehicles and Channels of Infection

The main vehicles of infection are the air we breathe, our food, and our drink.

Air-borne infection. Inspired air may contain two kinds of infective particles: *dust*, holding dried but still living bacteria; and *droplets* of saliva or mucus, coughed or sneezed out by an infected person. Small droplets dry while falling, and the contained microbes, only a proportion of which are killed by the drying, remain suspended for a long time and may be carried a considerable distance by air-currents. Large droplets, received direct from the source, naturally contain a larger dose of living microbes and are therefore the greatest menace of all.

The diseases spread by *droplet infection* are many and dangerous. Diphtheria, scarlet fever, meningitis, measles, influenza, pulmonary tuberculosis, whooping-cough, and colds by no means exhaust the list.

One of the difficulties in preventing the spread of infectious diseases is that infectiousness often precedes the onset of the illness. During the first day or two of measles, for example, the patient seems merely to have a cold, yet all the time he is spreading the virus abroad. Similarly, in whooping-cough the only symptom in the first week is an ordinary cough, yet the expelled droplets are laden with the causative microbe, *Haemophilus pertussis*. Thus it is towards the end of the 'incubation period'—the period when the microbes are multiplying and invading the tissues—that the spread of droplet-infection is at its height.

Infection by food. Cookery saves us from many evils, since the heat to which food is subjected is enough to kill most bacteria. But there are many risks of contamination between the cooking and the eating. Bacteria of the 'food-poisoning' group (*Salmonella enteritidis*; *Salm. typhimurium*, &c.) may be deposited in the food either by a 'carrier' (see below) among the kitchen staff, or by rats, mice or flies; and will multiply freely if the food is moist and warm. Outbreaks of gastro-enteritis with severe diarrhoea and vomiting are often due to contaminated food, especially meat-pies from inferior eating-houses. Another and more dangerous type of food-poisoning called botulism is due to *Clostridium botulinum*, which produces a violent poison during its growth in canned food.

Milk is a vehicle of many infections. Direct from the cow comes the bovine type of *Mycobacterium tuberculosis*, which lodges in the bones, joints, and lymph-nodes of children; and *Brucella abortus*, which sometimes causes a prolonged and troublesome 'undulant' fever. Moreover, milk is an excellent culture-medium for most bacteria, so that if a few pathogenic organisms are coughed into the pail or deposited in it by the

dirty hands of a milkman, they will multiply greatly before the milk is consumed; and since milk from various sources is often mixed in bulk, a widespread epidemic may result. Diphtheria and scarlet fever may be spread in this way by a 'carrier' (see below) among the dairy staff.

Water-borne infection. Streams and rivers may be contaminated by human and animal excreta from the surface of the land, or by sewage from dwellings. Leaking drains may discharge into a well or a reservoir, and if the drainage contains the discharges from a case of typhoid fever, cholera or some kindred disease, an epidemic is likely to break out.

Infection by human 'carriers'. After recovery from an infectious illness a small proportion of persons continue to 'carry' the living microbe for periods varying from a few weeks to a lifetime. After an attack of diphtheria or scarlet fever the microbes may persist on the surface of the mucous membrane of nose and throat, no longer able to invade the immunized tissues (p. 136). After typhoid fever the 'typhoid bacillus', *Salmonella typhi*, will sometimes survive and multiply for years in the gall-bladder, whence it is periodically discharged in the faeces. In dysentery chronic ulcers of the large intestine, compatible with fairly good health, may discharge *Bact. dysenteriae* for a long time. Finally there are certain bacteria, such as the meningococcus (*Neisseria meningitidis*), which are 'carried' in the nasopharynx of a considerable proportion of normal people. It may well be asked why these persons do not die of meningitis. As we shall see in Chapter XV, the explanation lies in the high *natural immunity* of most individuals to this organism, whereby any cocci that penetrate the mucous membranes are destroyed before they do any harm.

Insects as carriers of infection. Pathogenic microbes

of various species are disseminated by mosquitoes, flies, fleas, bugs, and lice. The plasmodium of malaria is sucked up by a mosquito with the blood of an infected man, and after undergoing a cycle of development in the creature's body it is transferred by another bite to a fresh human host.

Flies pick up bacteria and viruses on their legs from dung or offal, or they swallow and afterwards excrete them into our food. The intestinal infections, dysentery and typhoid fever, are often spread in this way.

The fleas of rats carry the microbe of plague (*Pfeifferella pestis*) from their dying hosts to human beings; and lice spread typhus fever in squalid and underfed communities.

Individual and Community Infection

When an infectious disease attacks a population we call it an *epidemic*. Many diseases break out periodically in epidemic form, and in the intervals the infection is kept alive either by a thin succession of scattered (*sporadic*) cases, or by carriers. Such diseases are said to be *endemic* in the community.

There is good evidence that in areas where an epidemic is raging many persons receive *subinfective* doses of the microbe, i.e. doses too small to overcome their *natural resistance*, but sufficient to inoculate them against subsequent infection. For example it has been shown that in a school which has suffered from recurrent epidemics of diphtheria many boys who have shown no symptoms of infection have nevertheless become *immune*. This is proved by their increased resistance to the toxin of *Corynebacterium diphtheriae* ('The Schick Test', p. 133).

Again, evidence of the acquisition of specific antibodies (Chapter XV) by individuals in contact with but not suffering from the disease has been obtained

during epidemics of typhoid fever, dysentery, and whooping-cough.

An epidemic, therefore, may be pictured as the temporary spread of a pathogenic microbe in a susceptible community, causing attacks of the disease in some individuals, and subinfection and immunity in others. Many individuals escape altogether, either by mere chance or because of an abnormally high natural or acquired immunity.

When a community has recently suffered an epidemic it is likely to remain for some time more infectious to new-comers than a normal community, because it will contain more carriers (latent community-infection).

The incidence both of individual and of herd-infection is affected by many additional factors, such as the frequency and size of the 'doses' of infection to which the susceptible individuals are exposed, the weather, and the general state of nutrition of the community. In insect-borne diseases like malaria the geographical distribution and local prevalence of the carrying insect play a central role.

Reaction; Recovery; Immunity

The symptoms of illness—fever, malaise, inflammation, rashes, and the like—are manifestations of the *reaction* of the tissues to irritation by microbes.

Some bacteria and viruses give rise almost invariably to *acute* illness which develops quickly, usually with fever and prostration, and runs its course in a few weeks. The infectious fevers, such as small-pox, measles, diphtheria and influenza, are instances of this type. The *chronic diseases*, such as tuberculosis and leprosy, tend to develop more slowly, and to last for months, years, or even a lifetime. Between these extremes there are intermediate states which we call

subacute. The manifestations of disease are, however, too variable to allow a precise classification on these lines. Acute illnesses may be prolonged into the chronic state, and microbes that generally cause chronic infection may sometimes give rise to the acutest of illnesses (e.g. *Myc. tuberculosis*). Moreover, a single species of microbe (e.g. *Streptococcus*) may cause a variety of generalized or localized infections of widely different chronicity.

Malaria, undulant fever and relapsing fever are examples of *recurrent infection* in which a series of acute attacks is punctuated by periods of comparatively good health. The different types of reaction to infection are the expression of the different kinds and degrees of irritation and damage caused by microbes of different chemical structure and biological habit. The diphtheria bacillus, for example, multiplies quickly in the throat and secretes a powerful poison (toxin) which causes an immediate and intense disturbance of vital functions. The *Mycobacterium* of leprosy, on the other hand, develops slowly, secretes no toxin, and gives rise at first only to a mild local irritation, which progresses slowly to disorganization and local death of the tissues.

Recovery from infective disease is due largely to the destruction of the microbes by *phagocytes*, i.e. leucocytes which first ingest and then digest them. This process is assisted by the antibacterial and antitoxic properties (antibodies) of the blood-plasma, which increase greatly during the course of the infection (Chapter XV).

Immunity. Recovery leaves the body in a state of heightened resistance, or 'acquired immunity', to the particular species of microbe concerned. After some diseases the immunity is weak and of short duration (e.g. influenza, colds); after others, like measles or small-pox, the protection is powerful and lifelong.

On recovering from an epidemic, a community appears generally to be immune for a time from further epidemics of the same kind. The degree and duration of the immunity probably depend on the number of individuals who have been immunized by infection or subinfection. With the gradual birth or immigration of new susceptible individuals the average level of immunity slowly sinks, and the population returns to its normal susceptibility.

It is, however, far from certain that acquired immunity is the sole cause of the wane of epidemics; indeed it is likely that in some cases a progressive loss of virulence of the microbe and other undetermined factors may play an important part.

Bacteriology, Medicine, and Research

Among the chief contributions of bacteriology to medicine is a broader conception of infective disease. Our attention is no longer centred exclusively on the individual patient and his symptoms, but widens out to a review of all the biological relationships of microbes to man and to the animal and vegetable kingdoms.

Moreover, a knowledge of the bacterial causes of disease and the vastly increased precision of diagnosis afforded by bacteriological methods permits a far more rational classification of diseases than one based on mere similarity of symptoms. We have learned, for example, that entirely different combinations of symptoms, which were formerly classed as separate diseases, may in reality be the variable manifestations of a single infection; and, conversely, what our predecessors held to be a single disease has in many cases proved to be due to several distinct bacterial causes.

Again, the study of *immunity* has given us a glimpse of the delicate and complex self-cleansing and self-

protecting mechanisms of the body, and we have learned ways of fortifying or supplementing these reactions by preventive and therapeutic immunization with serums and vaccines (p. 247).

The study of the properties and nature of viruses is in its infancy, and with improving methods of investigation it will continue to yield results of first-rate importance.

Chemistry, moreover, is continually refertilizing the soil of microbiological research, both by illuminating the inner processes of intoxication and immunity, and by the rapid extensions of Chemotherapy (p. 259), which bid fair to revolutionize the treatment of infections. A modern bacteriological research worker needs a much wider knowledge of the basic sciences than his predecessor; but with that equipment he has every expectation of rich rewards.

The Aims and Principles of Practical Bacteriology

Our first aim is *diagnosis*, i.e. the detection and identification of pathogenic microbes in suspected materials. The principles of procedure are as follows:

(1) Microscopic examination of pathological materials. Preliminary identification of the micro-organisms according to form and staining reactions.
(2) Pure-cultivation and determination of cultural characters.
(3) Examination of biochemical (fermentative) functions.
(4) Finer identification by serological tests (antigenic analysis).
(5) Tests of virulence and toxicity on animals.

The next three chapters deal with these procedures and the principles on which they are based.

Our second aim is the *treatment* of established in-

fections by specific means; viz. by preparing efficient therapeutic antiserums, or vaccines (p. 248) for injection into patients or by the administration of specific chemotherapeutic substances (p. 259). Finally, we aim at the *prevention* of infection, by checking its spread and by raising the resistance of those exposed to infection by active or passive immunization (Chapters XV and XVI).

Nomenclature and Classification

Until recent times bacteriologists have failed to agree on any proposed system of classification of bacteria into genera and species. They were content, for example, to call all rod-shaped organisms Bacilli, however widely remote in size, form and function. Similarly, all spheroidal cells were simply Cocci. The distinctive part of the name of a species was taken in one case from the discoverer, e.g. Bac. welchii; in another from the place of origin, e.g. Bac. melitensis (Malta), and in a third from the disease, e.g. Bac. pestis.

The more systematic and rational nomenclature adopted in this book arranges the various kinds of bacteria in as many broad groups or *genera* as there are clear and constant group-differences. In place of the indefinite 'Bacillus' welchii, melitensis, &c., we have *Clostridium welchii*, *Brucella melitensis*, *Pasteurella pestis* and so on, names which differentiate and place each of the species in a definite group. Some of the old names, however, are deeply rooted, and still in use in some countries; e.g. Bacillus tuberculosis or the tubercle bacillus for *Mycobacterium tuberculosis*. In this book only the modern names are printed in italics (e.g. pp. 18, 19).

Higher and lower bacteria. It is useful to be aware of this customary division of the Schizomycetes. The

higher bacteria are distinguished by growth in long, simple, or branched filaments. Very few of them are pathogenic to man (only *Actinomyces*, p. 156), but they include some interesting saprophytic organisms, such as the iron and sulphur bacteria, which utilize mineral substances in their metabolism and have certain features in common with the moulds (Hyphomycetes, p. 199). The great majority of pathogenic species belong to the lower bacteria. On pages 18 and 19 a list will be found which includes all the most important species with which we shall have to deal; a few others are mentioned at the end of Chapter XIII.

CHAPTER II

THE FORM AND STRUCTURE OF BACTERIA

Methods of observation. Bacteria may be examined with the microscope either alive, in a film of fluid between slide and coverslip (wet films), or dead and dried on a slide, after staining with various dyes (dry films). Of the two the wet film gives the more accurate picture of the natural form of the cell.

The staining of dried films. The most commonly used simple stains are 1 per cent. watery solutions of methylene blue, carbol-fuchsin, methyl violet, and carbol thionin blue. The dried film on the slide is fixed by passing it three times quickly through a Bunsen-flame, and is then treated for half to one minute with the stain. It is then washed, dried, and examined with the immersion lens; the immersion oil being put directly on the uncovered film. Most species of bacteria take up these dyes readily, but some, like *Mycobacterium tuberculosis*, need specially intensive treatment (p. 17). The compound stains of Giemsa or Leishman are excellent for films containing blood- or tissue-cells as well as bacteria, and they have the additional advantage of showing up spirochaetes and the malaria parasite, which are difficult or impossible to demonstrate with simple dyes.

Gram's double differential stain is based on differences in the physico-chemical structure of different species of bacteria. If a film is stained with methyl or gentian violet and then treated with a weak iodine solution, in some species the iodized dye combines so firmly with the cell-membrane that treatment with acetone or alcohol fails to dissolve it out; while in others it is easily removed. The former are called 'Gram-positive', the latter 'Gram-negative'.

The film, is treated after decolorization with a 'counter-stain' of dilute carbol-fuchsin or neutral red to colour the Gram-negative organisms red. Gram-positive bacteria appear deep violet or even black.

There is considerable variation in the tenacity with which the Gram-positive species retain the dye, and it is easy to be led astray by over-application of the solvent. Even with perfectly correct treatment certain feebly Gram-positive bacteria are reduced to a pale mauve.

Since Gram's stain is one of our most fundamental differential tests, lists of the main positive and negative species are given in Tables I and II.

The appearance of stained bacteria. In most species the cell stains more or less uniformly; but in some species (e.g. *Corynebacterium diphtheriae*) it tends to show alternate bands of light and dark, which indicates an uneven distribution of the chromatophilic (colour-loving) substance. Certain other species show 'bipolar staining', i.e. a deeper tint at the ends than in the middle. This is especially common in *Pfeifferella mallei* and *Pasteurella pestis.*

In any mass of bacteria some are always dead or dying, and their staining reactions are correspondingly altered. In Gram-positive films many Gram-negative elements may be seen. With methylene blue in dried films, dead cells and their remains take a pale purple tint, whereas healthy bacteria stain blue or greenish-blue.

Acid-fast bacteria. Certain species have the peculiar property of taking up hot carbol-fuchsin and holding it so firmly that they resist subsequent decolorization with strong mineral acids (Ziehl-Neelsen's method). This property seems to be due to a waxy alcohol in the cell-membrane. By far the most important acid-fast organisms are *Mycobacterium tuberculosis* and *Myco.*

leprae. No other human pathogenetic bacteria are acid-fast, but the saprophytic *Mycobacteria* (*Myco. smegmatis, phlei*, &c.) have this character, and also one or two animal-infecting species of *Actinomyces*.

TABLE I

The chief Gram-positive bacteria

Genus	*Species*	*Colloquial or old names*	*Disease caused in man*
Actinomyces	*Actino. bovis*	Streptothrix actinomyces; Ray-fungus	Actinomycosis
Mycobacterium	*Myco. tuberculosis*	Tubercle bacillus	Tuberculosis
	Myco. leprae	Leprosy bacillus	Leprosy
Corynebacterium	*C. diphtheriae*	Diphtheria bacillus	Diphtheria
	C. hofmannii, C. xerosis, &c.	Diphtheroid bacilli	Non-pathogenic
Streptococcus	*Str. pneumoniae*	Pneumococcus	Lobar pneumonia, peritonitis, &c.
	Str. pyogenes	..	Suppuration, scarlet fever, septicaemia
	Str. viridans	..	Endocarditis
Staphylococcus	*Staph. aureus, albus, &c.*	.	Suppuration, pyaemia, osteomyelitis
Micrococcus	*M. tetragenus*	..	Rarely suppuration
Sarcina	*Sarcina lutea*	..	Rarely suppuration
Bacillus	*Bac. anthracis*	Anthrax bacillus	Anthrax
	Bac. subtilis	Hay bacillus	Non-pathogenic
Clostridium	*C. tetani*	Tetanus bacillus	Tetanus
	Cl. botulinum	Bacillus botulinus	Botulism
	Cl. welchii	Bac. aerogenes capsulatus	Gas-gangrene

Dark-ground illumination, or '*the ultramicroscope*'. With ordinary direct illumination it is difficult or impossible to see very small or thin microbes, such as certain spirochaetes, Rickettsia, or the larger viruses. But the illumination can be so arranged, by means of a special sub-stage condenser, that the light only strikes

TABLE II

The chief Gram-negative bacteria

Genus	*Species*	*Colloquial or old names*	*Disease caused in man*
Pfeifferella	*Pf. mallei*	Bacillus mallei, or the glanders bacillus	Glanders
Pseudomonas	*Ps. pyocyanea*	Bacillus pyocyaneus	Suppuration ('blue pus')
Vibrio	*Vib. cholerae*	Comma bacillus	Cholera
Neisseria	*N. meningitidis*	Meningococcus	Cerebrospinal meningitis
	N. gonorrhoeae	Gonococcus	Gonorrhoea
	N. catarrhalis	Micrococcus catarrhalis	Nasopharyngeal catarrh
Proteus	*Pr. vulgaris*	Bac. proteus vulgaris	Suppuration
Bacterium	*Bact. coli commune*	Bacillus coli	Occasionally suppuration, cystitis and pyelitis
	Bact. friedländeri	Pneumobacillus or bacillus mucosus capsulatus	Occasionally pneumonia ?Rhinoscleroma
	Salm. typhi	Typhoid bacillus	Typhoid fever
	Salm. paratyphi (*A*, *B*, and *C*)	Bacillus paratyphosus, &c. (Salmonella group)	Paratyphoid fever, gastroenteritis (food-poisoning)
	Salm. enteritidis (*Gaertner*)		
	Salm. typhimurium		
	Bact. dysenteriae (group)	The dysentery bacilli	Bacillary dysentery
Pasteurella	*Past. pestis*	Bacillus pestis	Plague
Haemophilus	*H. influenzae*	Pfeiffer's bacillus	Catarrhal inflammation
	H. pertussis	Bordet-Gengou bacillus	Whooping-cough
Brucella	*Br. melitensis*	Micrococcus melitensis	Mediterranean fever
	Br. abortus	Bacillus abortus of Bang	Undulant fever
Spirochaetes	All species	..	Syphilis, ictero-haemorrhagic jaundice, &c.

the film (which must be a wet one) obliquely from the sides. Bacteria and other particles in the film scatter or 'diffract' the light and are seen as brilliant images

on a dark background. In addition to a special condenser, a more concentrated light is essential. A 'stop' or an iris diaphragm is needed for fitting into the ordinary immersion lens, and some immersion-oil must be put between the slide and the condenser. Alternatively, the high-power dry lens may be used with a strong eyepiece, (see also p. 207).

Morphology

Bacteria consist of a protoplasm surrounded by a thin, flexible *membrane*, probably composed of proteins and lipoids. The writer has seen a living cell enter through a rent in the ballooned membrane of a dead one, and swim about inside. Hypertonic fluids sometimes cause 'plasmolysis', or shrinking of the protoplasm away from the membrane.

Intracellular structures. Organized *nuclei* comparable with those of protozoal or metazoal cells are not found in bacteria. It is, however, an open question whether certain intracellular bodies which stain deeply with basic dyes may not represent a primitive nuclear apparatus. Bodies of similar size and shape have been demonstrated by photography with ultra-violet light, and there is some inconclusive evidence of their fission preparatory to the division of the cell. But it is significant that they do not take the haemotoxylin stains, which have so strong an affinity for nuclear chromatin, and there is no sure way of distinguishing them from the highly refractile and deep-staining *intracellular granules* contained by many bacteria. These latter are variously termed (1) 'Metachromatic granules', from the fact that their colour in stained preparations differs somewhat from the rest of the cell; (2) 'Ernst-Babes granules', after the two bacteriologists who first described them; or (3) 'Volutin granules', which signifies no more than that the

obscure substance of which they are composed has been given that name. Though often mistaken for nuclei, these objects are not permanent structures but appear to be fluctuating food-reserves. The largest of them may give the chemical reactions of lipoids or starches. Finally, degenerate or dead cells often show deep-staining granules due to the break-up of the protoplasm. These, when liberated from swollen, dying cells through a burst membrane, are liable to be mistaken for special reproductive bodies, or gonidia; but they do not germinate when planted on fresh medium.

Flagella and Motility

Many of the rod-shaped bacteria have one, two, or more exceedingly delicate vibratile appendages known as 'flagella', commonly at one or both poles of the cell. The undulation of the flagella makes currents in the surrounding fluid, whereby nourishment is brought to the cell, and its waste products removed. When the cell is floating in liquid, the flagellar vibrations drive it along with a rotary movement. Such species are called 'motile'. The various arrangements of flagella with their descriptive terms are given in Fig. 2. These organs are invisible with the ordinary microscope, and can only occasionally be seen by dark-ground illumination. Special staining methods are needed to demonstrate flagella in dried films. Good preparations are hard to make, and the appearances may be very deceptive. The number of flagella possessed by the various species is by no means certain in all cases. Even up to 1930 *Salmonella typhi*, one of the best-investigated species, was universally believed to be peritrichate, and *this is still the orthodox view*; but recent work indicates that it really has only one flagellum on each side (Fig. 14, p. 79). Further

research on this and other supposedly peritrichate species is desirable.

Observation of motility. A wet unstained film of a fluid culture not more than 24 hours old is examined with the high-power dry lens. True motility consists

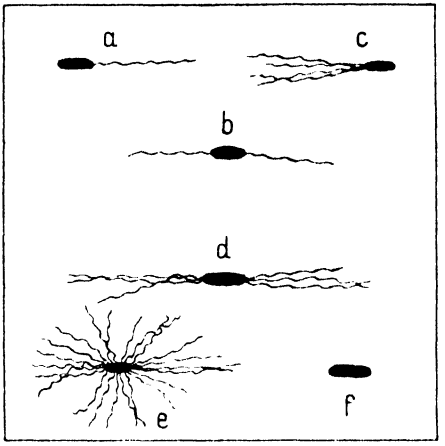

FIG. 2. Flagella

a. Monotrichate. b. Amphitrichate. c and d. Lophotrichate. e. Peritrichate. f. Non-motile (atrichate).

in a definite progression of cells in various directions relative to neighbouring cells or other objects. It must be distinguished from *Brownian movement*, in which the cells merely jerk about under the influence of molecular bombardment without much change of position; and also from *streaming with a current*, which betrays itself by the parallel movement of many cells in one direction. Young cultures on solid media can also be used for the detection of motility, but the results are less reliable.

Variation of flagella. In most motile cultures there are many non-motile individuals. Moreover, certain species produce variant races without flagella. In the genus *Proteus* the motile variant spreads in a thin film all over the surface of solid culture-media, whereas the non-motile variant forms ordinary colonies. The motile variant is called the H form, from the German 'Hauch' (film), while the non-motile type is called O ('Ohne Hauch'; without film). The variation is nearly always transitory, the race soon reverting to its normal state, but permanent variations (mutations) of this type are known to occur in other motile species (*Salmonella typhi* and *typhimurium*). In spite of the absence of film-formation in these latter species, the terms H and O are applied to the motile and the non-motile variants respectively (see also p. 81).

TABLE III

The chief motile bacteria

Genus	*Species*
Vibrio	All species including *Vib. cholerae*
Pseudomonas	All species including *Ps. pyocyanea*
Proteus	All species including *Pr. vulgaris*
Bacterium	Many species including *Salm. typhi; paratyphi (A, B, C); enteritidis (Gaertner); typhimurium; Bact. coli* (some varieties non-motile)
Bacillus	Several non-pathogenic species including *Bac. subtilis*
Clostridium	Several species including *Cl. tetani* and *Cl. botulinum*
Spirochaetes	All species

Significance of flagella. It is curious that the flagellate bacteria seem to be no more successful in the struggle for existence than the unflagellate. Motility certainly has no connexion with the invasion of tissues,

which, as we have seen (p. 6), is a passive phenomenon, nor with pathogenicity in general. Seeing that most soil- and water-saprophytes have flagella, we may surmise that these organs are more useful in the outer world, where nourishment must usually be scanty and dilute, than in the body, where it is profuse and concentrated.

Capsules. In a few species of bacteria the cell is surrounded by an ectoplasmic envelope (Figs. 9 & 13). In ordinary stained films it appears as an unstained halo, but it can be coloured by special staining methods such as Muir's capsule stain (Muir and Ritchie, *Manual of Bact.*, 10th ed., p. 123). *Streptococcus pneumoniae* (pneumococcus) and *Bacterium friedländeri* form capsules both in the tissues and in cultures; *Clostridium welchii* and *B. anthracis* form them regularly in the tissues only. *Str. pyogenes* and some other species may or may not show them in the tissues. In certain cultural conditions a normally capsulated species may produce a temporarily unencapsulated growth or even give rise to permanently unencapsulated variants.

Capsule-formation in general seems to be a defensive mechanism of the bacteria against the bactericidal action of phagocytes and antibodies. The capsular material is a mucilaginous secretion of varying firmness rather than a permanent structure. In numerous species it is known to be rich in polysaccharides, which play a very important part in the stimulation of the tissues to specific antibody formation (p. 238).

Spore-formation. Bacteria of the genera *Bacillus* and *Clostridium* have the power of passing into a highly resistant resting phase by the production of thick-walled spores (or endospores), rich in lipoids and poor in water. Since a single cell produces only a single spore, the process is evidently not reproductive.

Sporulation generally occurs when nourishment has

been profuse but is becoming either exhausted or inaccessible owing to desiccation. As soon as the spores find themselves again in warm, moist, and nourishing surroundings they germinate into fresh vegetative cells, leaving their empty membranes behind.

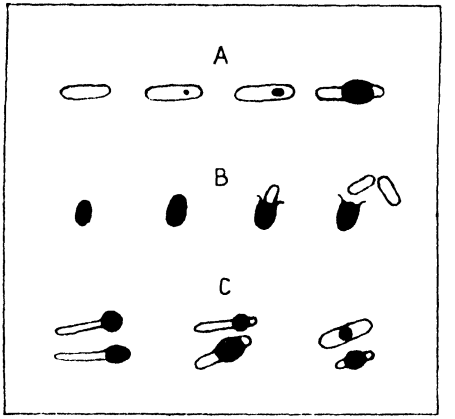

FIG. 3. Spores

A. Stages in the formation of a spore.
B. Stages in the germination of a spore.
C. Positions of spores. Terminal (left); Subterminal (middle); Equatorial (right). Above, spherical. Below, oval.

The formation of a spore (Fig. 3) begins with the appearance of a granule or of a collection of granules of deeply staining matter at some point in the interior of the cell. This enlarges into a deeply staining mass surrounded by a clear halo. Finally, as soon as the halo has materialized into an impermeable spore-membrane, the spore itself refuses to take up the stain at all. In wet unstained films the spores show up as highly refractile spheroids, either lying free or still contained in the cell (sporangium).

For colouring spores special methods are needed, of which one of the simplest is to stain for 5 minutes with carbol-fuchsin, decolorize with ½ per cent. sulphuric acid, or with alcohol, and counterstain with methylene blue.

The site of the spore differs in different species. The expressions 'terminal', 'subterminal', and 'equatorial' (Fig. 3) explain themselves. Those species in which the spore is wide enough to cause a bulge in the containing cell are called *Clostridia*; those in which no bulge is seen are termed *Bacilli*.

Spores have no special pathogenic function. The great majority of spore-bearing species are, in fact, saprophytes, and it is clear that the survival-value of the spore lies in its tolerance of light, heat and desiccation in the outer world. *Variation* of the spore-forming faculty has been observed in several species. Completely asporogenous varieties of *Bacillus anthracis* have been obtained by growth at 42° C. and by other procedures.

Chemical composition. Like all living cells bacteria contain proteins, nucleo-proteins, lipoids, carbohydrates and mineral ash, which may contain anything from 10 per cent. to 70 per cent. of phosphoric acid. The relative quantities of the components of the cell vary considerably with the composition of the culture-medium. The capsular substance of the pneumococcus and numerous other species is, as we have already seen, rich in polysaccharides.

There is increasing evidence that the membrane is composed of a mosiac of different chemical groups to which the specific actions and reactions of the bacteria are largely due.

Considerable knowledge of the enzyme-actions of bacteria, chiefly nonpathogenic, has accumulated and can be studied in special treatises on bacteriological chemistry.

Antigenic Structure

By virtue of the protein or protein-carbohydrate constituents (antigens) of the membrane or deeper parts of the cell, bacteria or their products when injected into animals cause the production of specific antibodies. These can be detected and measured in various ways, e.g. by the *agglutination* (clumping) of the bacteria in suspension or by the *precipitation* of bacterial extracts.

The agglutination reaction. If the blood-serum of an animal which has received a suitable course of injections of living or dead bacteria is mixed with a suspension of the same species of microbe, the cells clump together into visible aggregates. This can be demonstrated either by suspending some bacteria from a solid medium in a loopful of serum on a slide, or by mixing the serum with a broth-culture or saline suspension (live or killed) in a tube. A quantitative measurement of the 'agglutinating power' of a serum is made by testing progressive dilutions of the serum with a constant volume of suspension, and noting the highest dilution in which the serum reacts. This is called its 'titre'. The agglutinin (i.e. agglutinating antibody) produced in response to a given bacterium is generally complex, for the antigenic structure of bacteria is seldom, if ever, simple. As a rule the major part of the antibody is *specific*, that is, capable of reacting only with the one species of microbe; but a minor fraction often shows affinity for related species of the same genus, thus giving evidence of the existence of common or 'group' antigens. The degree to which specific and group antibodies are produced varies with different species and also in some cases with different phases or states of a single species (p. 82). Generally speaking, a given bacterium reacts more

rapidly and completely with its own (homologous) antiserum than with other (heterologous) antiserums, however closely related.

In the motile bacteria the position is complicated by the fact that the flagella contain antigens additional to, and distinct from those of the cell-body (somatic antigens). The flagellar antigens, owing to their association with motility, are called H antigens (p. 23), while the somatic antigens, being the only ones present in non-motile variants, or in motile ones whose flagella have been destroyed by heat (100° C.) or alcohol, are called O antigens. The agglutinins elicited by these different antigens are similarly termed H or O agglutinins. An antiserum against a normal motile culture will contain both flagellar (H) and somatic (O) agglutinins, and the agglutination it causes will be a mixture of floccular, flagellar agglutination and granular, somatic clumping. Serums made with suspensions devoid of flagellar (H) antigens will cause only O type granular clumping, even in flagellated suspensions.

The agglutination reaction is extensively used in the identification of bacteria grown from pathological material. Diagnostic laboratories keep ready a range of specific agglutinating serums for this purpose. Moreover, since infection gives rise to specific antibodies, a patient's serum will, in certain diseases, agglutinate suspensions of the infecting microbe, and no other. The most famous test of this type is the *Widal reaction* for typhoid fever (p. 87), and similar tests are now employed in the diagnosis of the paratyphoid fevers, gastro-enteritis, Mediterranean and undulant fevers, cholera, and bacillary dysentery.

An extension of the agglutination reaction, known as the *absorption test*, is useful for distinguishing two species which, owing to a partial community of anti-

gens, cannot be separated with certainty by the simple agglutination test. The principle of the test is as follows: if a serum containing, let us say, antibodies A and B is mixed with a sufficient mass of bacteria containing antigen *a*, but no *b*, the bacteria will combine with antibody A, leaving B free, so that if the mixture is now centrifugalized to remove the bacteria with the combined antibody, the supernatant fluid will be found to contain antibody B alone; that is, it will only agglutinate bacteria possessing antigen *b*. To take an example, *Salmonella paratyphi B* and *Salm. typhimurium* have common antigens (H and O) in addition to the specific factor (H) of each. Their respective antiserums therefore contain both common and distinctive agglutinins. If each serum is 'absorbed' with each bacterium separately, the following results will be obtained (Table IV):

TABLE IV

Treatment of serum	*Tested on H suspensions of:*	
	para. B	*typhi-murium*
Serum paratyphi B unabsorbed	+	+
,, ,, absd. with *S. para. B.*	0	0
,, ,, absd. with *S. typhimurium*	·	0
Serum typhimurium unabsorbed		+
,, ,, absd. with *S. para. B.*	0	+
,, ,, absd. with *S. typhimurium*	0	0

Note. + = Agglutination; 0 = No agglutination.

Each species removes *all* the agglutinins from its homologous (same type) serum, but only the common agglutinin, from the heterologous (different type) serum. If, therefore, we wish to identify a bacterium of this group, which we have found to be agglutinable by both the serums, we saturate them both with it and test the reactions of the absorbed serums in

the manner shown. By this means we can determine the species with which our bacterium corresponds in absorbing power.

The precipitation reaction is fundamentally like agglutination, but differs in that the phenomenon observed is the clouding and subsequent precipitation of mixed colloidal solutions of antigen and antibody (p. 241). The bulk of the precipitate is serum-globulin, and it is therefore best to work with a constant volume of serum and progressive dilutions of antigen instead of the converse arrangement which is usual in the agglutination test. Antigenic analysis can be carried a stage further by this reaction, using antigenic fractions chemically extracted from bacteria and precipitating antiserums prepared by injecting rabbits with the fractions.

When many races of any genus are analysed by agglutination and precipitation they tend to show the following general arrangement of their numerous antigens: one or more antigens common to the whole genus; one or more to larger or smaller groups (subgenera); one or more to individual species, and one to each serological type or variety within the species. These various antigens are accordingly called *common, group-specific, species-specific,* or *type-specific.*

The broader serological groupings nearly always tally with the grouping by some cultural or biochemical character, whereas the serological types are only distinguishable by serological means.

Dimensions. Variation. Involution

The length of most bacteria lies between 1 and 10 μ and their width between 0·2 and 1 μ (N.B. 1 $\mu = 0{\cdot}001$ mm.; cf. p. 209). Most cocci are about 1 μ in diameter. Although each species of bacteria has a specific average form and size, the individual cells may vary

greatly in length. Thus long filaments, apparently due to fission lagging behind growth, are not uncommon in the rod-shaped species.

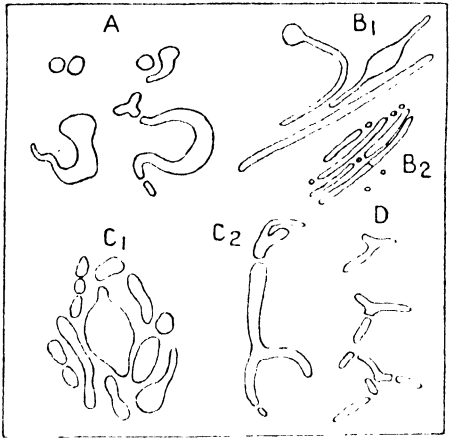

FIG. 4. Involution forms and abnormal growth

A. Four stages in the development of a coccoid involution-form of *Vib. cholerae* into a larger irregular and branching form. The second coccoid underwent autolysis (direct continuous observation on an agar surface).

B_1. Filamentous and ballooned cells of old stock culture of *Vib. cholerae*. B_2. Sterile small 'coccoids' in same culture.

C_1. Abnormal race of *Bact. coli*, growing in ballooned and distorted forms. C_2. Grotesque lobster-like branched form in same culture.

D. Three stages in the production of normal rods from a branched or Y-form of a bacterium (direct continuous observation).

In the early stages of growth in fresh culture-medium the cells are generally larger than in mature cultures when overcrowding has put a stop to multiplication. In old, exhausted cultures one may often see swollen, elongated, bulbous or branched cells,

which are termed 'involution forms' and indicate a disturbance of growth due to starvation or toxic agents (Fig. 4). When planted on fresh medium many of these cells show no sign of life, but others will grow and multiply, giving birth either to a normal race or more rarely to a race of permanently altered shape. In the latter case we seem to be dealing with a mutation by the loss of a genetic factor governing normality of form. Bacteria that produce numerous involution-forms in comparatively young cultures are sometimes termed *pleomorphic*.

CHAPTER III

THE CULTIVATION OF BACTERIA

Objects and Methods

For proper examination bacteria must be obtained in *pure culture*. If microscopic examination of the material shows only one kind of microbe the introduction of a fragment or droplet into a tube of sterile nutrient liquid, such as meat-broth, will generally suffice; but even here a few bacteria of another kind may have escaped notice, in which case a *mixed culture* will result.

The classical method of isolating each species from a mixture is known as *plating out*. A droplet of the material (e.g. pus, blood, mixed culture, &c.) is spread on the surface of a stiff nutrient jelly (agar or gelatin, p. 40) in a flat, covered Petri-dish (Fig. 5). In the course of 18 to 24 hours in the incubator a crop of colonies will appear, each derived from a single cell or group of cells; and it is generally possible to judge from their appearance whether one, two, or more species of bacteria are present. After clinching the matter by microscopical examination, a pure culture of each type is made in a separate culture-tube. This is done by touching the colony with the tip of a sterile platinum needle (Fig. 5) and then dipping the needle into a tube of liquid culture-medium, or streaking it over the surface of a solid medium. Having thus obtained pure cultures of our microbes we can proceed to examine their *cultural characters* by *subcultivating* them in a number of different media and observing the quality and quantity of growth under various conditions of temperature, oxygen pressure, and so on. In some cases we next determine the *fermenting powers*

of the organism on a number of carbohydrates (p. 40), and finally we prepare suspensions for *serological examination* (p. 27) and *virulence tests* (p. 47), either by washing off the growth from solid media with saline

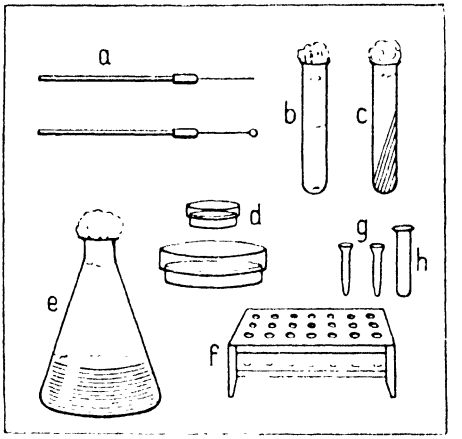

FIG. 5. Indispensable bacteriological tools and apparatus

a. Platinum or nichrome needle and loop. b. Culture tube of fluid medium or of solid medium for stabs. c. Agar or gelatin slope-tube. d. Petri-dishes. e. Erlenmeyer flask. f. Zinc rack for serological tests. g. Agglutination-tubes for serological tests. h. Dwarf test-tube for making dilutions.

solution, or merely by growing the organism in a fluid medium.

Bacteriological technique—sterility. An object or material is said to be sterile if it is entirely free from living organisms. But since microbes are ubiquitous, nothing may be assumed to be sterile unless it has just been sterilized. And even then the slightest touch of an unsterile object, or a few seconds exposure to the air, may contaminate it.

Methods of sterilization in general will be considered in Chapter XVI. Bacteriological tools (platinum-needles and loops, Fig. 5) are heated to redness in a naked flame immediately before use. Before opening a culture-tube that has stood exposed to the air for any length of time the wool plug should be set on fire for a moment to destroy any bacteria that may have settled on it; otherwise in opening the tube they may be dislodged and contaminate the medium. The skin of the hands, even after washing, is bacteriologically unclean, and it is therefore best to use flamed forceps wherever possible instead of fingers. Bacteriological procedures need *still air*: draughts, especially in towns, bring dust and spores.

The invaluable function of cotton-wool as a porous barrier against contamination depends on the fact, which was demonstrated by Pasteur, that bacteria do not travel along a narrow channel of stationary air, but sink and adhere to its walls. Since air passes through the tortuous passages of a wool plug by diffusion, without appreciable currents, all bacteria are arrested in the outer layers. Plugs must, however, be kept dry, since mould-spores are capable of germinating in damp wool and pushing their mycelial threads right through into the tube.

Nutrition and Conditions of Growth

Like other living things, bacteria need water, carbon, oxygen, and nitrogen; also traces of sulphur, phosphorus, and other inorganic elements.

Many soil-saprophytes can obtain C and N from inorganic sources; C from CO_2 or CH_4; N from NH_3 salts or, in some cases, from air. Not being dependent on organic matter from other living things, they are termed *autotrophic*.

Pathogenic bacteria need organic compounds. They

obtain nitrogen most easily from peptones and amino-acids; few of them can digest unaltered proteins. Carbon is acquired by the fermentation of carbohydrates and organic salts. Energy is obtained, as in larger creatures, by the oxidation of carbon; in other words bacteria show a respiratory exchange of oxygen and CO_2. Some pathogenic species have been proved unable to synthesize all their necessary enzymes and catalysts from simple nitrogenous compounds. *Staphylococcus aureus*, for example, needs nicotinic acid as a kind of vitamin, and the *Haemophilus* group need haematin. It is likely that all pathogens have some such requirements.

Aerobiosis and anaerobiosis. According to their method of obtaining oxygen, bacteria are divided into *aerobes*, which thrive in air, and *anaerobes*, which can only multiply in the absence of gaseous oxygen. The latter obtain their oxygen by the fermentation of organic substances. Some aerobic species are *strictly aerobic*, but many can live anaerobically if forced to do so, and are therefore termed *facultative anaerobes*. Among the bacteria that will not grow in air some are *strictly anaerobic*, i.e. intolerant of the least trace of O_2; and others are merely *micro-aerophilic*, i.e. grow best in reduced oxygen-pressures.

We have recently learned, however, that an even more fundamental condition for anaerobiosis than the presence or absence of O_2 is the reducing-power of the medium as a whole. This can be measured electrometrically and is more correctly termed the *Oxidation-reduction potential* or *Eh* of the system. If this potential is low enough anaerobes will germinate and multiply even if O_2 is present in the atmosphere.

Anaerobic cultivation. A low enough potential is, for example, present in deep tubes of meat-mince cooked in a little water; though in most media it can

only be produced by strict exclusion of oxygen. This may be done by enclosing the culture in an airtight vessel with a mixture of pyrogallic acid and KOH to absorb the oxygen. But more perfect anaerobiosis is obtainable with a special anaerobic jar, e.g. that of Fildes and Macintosh, in which the air is exhausted with a pump and replaced with hydrogen, the last traces of O_2 being 'burned' away by an electrically heated element of platinized asbestos. It is always best to use freshly heated, and therefore airless, media. Deep tubes of agar or glucose-agar, autoclaved just before use and cooled to 50° C., are used for making 'shake-cultures', the material for cultivation being planted deep into the medium and then dispersed by rolling the tube between the hands. Even if traces of O_2 remain in the atmosphere growth will probably occur in the depth of the tube.

The most important anaerobes are *Clostridium tetani*, *Cl. welchii*, *Cl. botulinum*, and other species of that genus, which are classed together as the *spore-bearing anaerobes*; also *Actinomyces bovis*. Most spirochaetes, e.g. *Treponema pallidum* are micro-aerophilic.

Moisture is necessary for growth. Desiccation causes a high death-rate of vegetative cells, though a proportion of them may survive indefinitely. Cultures are nowadays often preserved by desiccation in vacuo over phosphorus pentoxide. Spores are especially resistant to drying.

Temperature. Pathogenic microbes grow best at about the temperature of the body, 37° C., but the majority will multiply at anything between 20° and 43° C. The best temperature for most saprophytic bacteria is about 20° C. Most bacteria survive *cold*, even down to −250° C., and a number of saprophytes, with *Pseudomonas pyocyanea*, will multiply at 0° C. Such species are termed *psychrophilic* or cold-loving.

Heat is far more harmful. The growth-rate of nearly all species falls off rapidly above 40° C. and ceases at 43°–45° C. The only exception to this rule is a group of *thermophilic* saprophytes, which are found in milk, manure, and in the water of hot springs. These multiply freely at 60°–70° C., which kills all ordinary bacteria. The sterilizing action of heat is considered on p. 255.

Light. Direct sunlight is fatal in a few hours to the vegetative forms of bacteria; though they can stand diffuse light for long periods. For rapid multiplication, however, darkness is essential. It is the ultra-violet end of the spectrum that does the most damage.

Osmotic pressure. Bacteria are more tolerant than other unicellular organisms to changes of osmotic pressure. They may survive for a long time in pure water, though there is always a steady death-rate from starvation. Survival is longest in weak solutions of mixed salts, such as Ringer's fluid. At stronger concentrations salts at first inhibit growth and then destroy life. Generally speaking, the higher the atomic weight of an element, the more toxic are its salts.

Hydrogen-ion concentration (*pH*). This can be directly measured by means of a special electrical potentiometer with a hydrogen electrode, but in practice the colour-response of dyes such as phenol red, neutral red, and litmus to changes of reaction is used as a sufficiently accurate measure.

Generally speaking, the best pH for the growth of bacteria is slightly on the alkaline side of neutral (7·0), i.e. between 7·4 and 7·6. Most species grow poorly at an acidity beyond 6·0 or at an alkalinity beyond 7·8. At about 5·0 and 9·0 respectively growth ceases and gradual death ensues. In cultures of many bacteria acids are produced from the carbohydrates, and ammonia from the protein derivatives in the medium. If

fermentable carbohydrate is added in sufficient quantity the acid outstrips the alkali, and the acidity may rise high enough to sterilize the culture.

Liquid Culture-media

Nutrient broth consists of infusion of beef, veal, or horse-flesh, enriched with peptone and sodium chloride. The pH, which is always on the acid side, is titrated with NaOH solution and an indicator such as phenol-red, and then adjusted to 7·6 or any other desired value by the addition of the calculated quantity of NaOH. The broth is distributed in wool-plugged test-tubes or Erlenmeyer flasks (Fig. 5), and sterilized in the autoclave (p. 256).

For the cultivation of ordinary robust bacteria, meat-extracts such as Lemco may be substituted for the infusion.

The character of bacterial growth in broth varies according to the species of the bacterium, and may take any of the following forms: uniform turbidity; flocculer or punctate growth falling to the bottom of the tube; or pellicle-formation, i.e. a thick film on the surface.

Trypsinization and enrichment. The addition of trypsin to broth assists the growth of delicate organisms such as the meningococcus by breaking down albumoses and peptones into amino-acids. Similarly, the addition of a little blood, serum, or ascitic fluid greatly assists the development of many organisms.

Peptone-water. A 1 to 2 per cent. solution of peptone in 0·5 per cent. NaCl solution is used as a sugar-free basis to which various carbohydrates may be added for testing the fermenting-powers of bacteria. It is also used, without any addition, for the detection of indole-production, which is an important criterion for the identification of species.

The test for indole is carried out by incubating a peptone-water culture of the bacterium for three days; pouring a few c.cm. of ether into the tube; agitating to mix thoroughly, and then running a few drops of Ehrlich's reagent (paradimethylaminobenzaldehyde) gently on to the layer of ether which rises to the top of the tube. A purplish red ring indicates the presence of indole.

Fermentation-reactions. Carbohydrates or alcohols such as glucose, lactose, mannitol, saccharose, maltose, dulcitol, or xylose are added to peptone-water to make a 1 per cent. solution; a dye such as phenol-red being added to indicate acid-production. At the bottom of each tube of medium is put a miniature inverted tube to demonstrate the production of gas. If fermentation takes places at all the change of reaction is usually visible after a night's incubation; but a delay of anything from one to three weeks is not uncommon in certain species (pp. 80 and 98).

Milk, with litmus or phenol-red as indicator, serves to distinguish bacteria that cause acidification and coagulation from those that do not. It must be fresh and of good quality; but even so its composition is variable, and the effects of a bacterium on different samples is not perfectly constant. Many bacteria produce both acid from the carbohydrates and alkali from the protein-constituents, and the pH, which may change from acid to alkaline during the growth of the culture, is the resultant of the two processes.

Solid Culture-media

Nutrient agar. The basis of this medium is a gelatinous substance, agar-agar, extracted from Oriental seaweeds. It is added to nutrient broth in sufficient quantity (1½ per cent.) to make a stiff jelly. The agar itself is not nutrient, but merely serves as a stiffening

for the broth. The properties that make it so useful in bacteriological work are its high melting-point (95° C.) and a low solidification-point on cooling (about 42° C.). It thus provides a solid medium for the cultivation of bacteria at 37° C. or over, which is above the melting-point of gelatin (see below). For use, the melted medium is poured into test-tubes, which, after sterilization in the autoclave, are either propped on the slant so that the agar sets with a long flat surface (*slopes*), or upright, to obtain a deep column of medium for stab-cultures (see Gelatin, below). For plating out mixtures some melted sterile agar is poured into sterile Petri-dishes to form a layer about a quarter of an inch deep.

Enriched agar. For the growth of certain bacteria an *enrichment* of the agar in one or other of the following ways is necessary. *Serum agar*: 5 to 10 per cent. of sterile serum is added to melted nutrient agar, after cooling to 50° C. *Ascitic-fluid agar* is made in the same way. *Blood-agar* can be prepared either by adding one or two drops of sterile blood to each c.cm. of melted agar (cooled to 50° C.), or by smearing a little blood on the surface of agar slopes. For *Haemophilus influenzae* and some other organisms blood-agar may be heated to 80° C. for a few minutes, or boiled for a few seconds, without destroying its stimulating property. Other types of enrichment are useful in special cases, e.g. glucose, glycerin, potato- or pea-flour extracts.

Nutrient gelatin is made in the same way as nutrient agar; but since gelatin melts at 25°–30° C. it cannot be used as a solid medium at temperatures much above 20° C. Its chief use is for determining whether a bacterium produces proteolytic ferments that liquefy the medium. This phenomenon is best seen in *stab-cultures*, i.e. cultures made by stabbing the platinum-needle, loaded with bacteria, down the centre of a tube of the

medium. After a few days at 20°–25° C. a cup of liquefaction is seen, spreading down and out from the point of needle-puncture.

The main pathogenic gelatin-liquefying bacteria are:

Staphylococcus	*Proteus*
Vibrio cholerae	*Bacillus anthracis*
Pseudomonas pyocyanea	*Clostridium tetani, welchii, &c.*

Potato. Flat-faced wedges are cut from cleaned and peeled potatoes, soaked in a weak alkaline solution to correct their natural acidity, and put into special large tubes. These are plugged with wool and sterilized in the autoclave. This medium is chiefly used for *Mycobacterium tuberculosis.*

Egg. The common type consists of the mixed whites and yolks shaken in a flask with some sterile 0·9 per cent. NaCl solution or broth, distributed into tubes and solidified on the slope in an open hot-air-oven (inspissator) at about 80° C. It is especially useful for *M. tuberculosis* and *N. meningitidis.*

Solid serum. Ox or other blood-serum heated to 80° C. in an inspissator makes an opaque whitish solid medium especially suitable for the isolation of *Corynebacterium diphtheriae* from the throat.

Growth and Multiplication

Fission. Bacteria multiply by simple transverse fission. When the cell has reached the maximum size appropriate to the species a constriction appears at its centre, the membrane grows inwards and forms a septum which finally splits across, releasing the new twin cells. These may or may not immediately separate; in some species they tend to adhere for a time in pairs (diplococci; diplobacilli). Abnormal fission may occur in rod-shaped species when an old culture is revived with fresh nourishment. Some of the 'involution-forms'

(p. 31) grow into bizarre, swollen cells or long threads, which occasionally sprout in three directions, producing Y-forms capable of generating new normal cells at each of their three growing-points ('Three-point multiplication').

Colony-formation. The colonies of the different genera of bacteria are generally fairly characteristic, but those of different species within a genus may be very much alike. The following characters should be observed: size; colour; opacity or translucence; body of colony flat, evenly domed, bossed, or depressed in the centre; consistency hard, soft, or sticky when touched with a needle; surface smooth and shiny or rough and dull; edge sharp and evenly curved or wavy and irregular.

Colony-variation. Smooth and rough phases. Although the colonies of a given species are generally fairly uniform, a certain variability is to be expected, and in some cases the differences are so great as to give rise to a suspicion that the culture is impure. An interesting type of variation consists of the production of rough colonies with wavy edges in cultures which have previously shown only smooth, even colonies (Fig. 6). The phenomenon is best shown by the genus *Bacterium* (*Bact. coli*, *Salm. typhi*, &c.), but it occurs in different degrees in most, if not all, genera. The change in the organism, to which the colony-variation is due, consists of the loss of a chemical constituent or 'antigen'. This is generally accompanied by a great diminution of virulence. The reduced, rough variant is very sensitive to the flocculating action of electrolytes, and therefore grows in ordinary salty fluid media in the form of clumps and flakes which settle to the bottom of the tube. In some bacteria (e.g. *Haemophilus* and *Brucella*) the same fundamental antigenic reduction occurs, but without any obvious 'roughening'. In these cases it is

detected by agglutination and absorption tests (p. 27) with anti-smooth and anti-rough serums.

The rough variation occurs by far the most often in races of bacteria that have lived for some time under 'domestication'. The smooth antigen, on which viru-

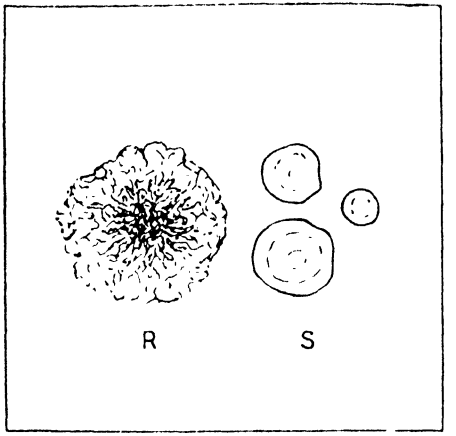

FIG. 6. Rough (R) and Smooth (S) Colonies (Genus *Bacterium*)

lence depends in most species (p. 48), seem to be no longer necessary for growth in culture, and its loss may be interpreted as an adaptation to a saprophytic type of life in culture-media. The change does not generally occur all at once, but in steps, and is reversible in its early stages. It can also generally be induced by cultivating the smooth type in media containing specific antiserum—a curious fact, of which we do not know the full significance.

Many other interesting forms of variation have been recorded, some of which are accompanied by striking

colony-differences: pigmentary changes, giving colourless colonies in a pigmented species; loss of motility or of the power of spore- or capsule-formation. All of these may be either temporary or permanent. A curious alternating variation of antigenic structure will be described in connexion with the Salmonella group (p. 81).

CHAPTER IV

PATHOGENIC ACTION

THE principles of proof that a particular species of microbe is the cause of a given disease are embodied in a set of 'postulates' which we owe to the school of Robert Koch.

Koch's postulates. (1) The microbe must be found in all cases of the disease, and its distribution in the body must correspond with that of the lesions.

(2) It must be grown in successive pure cultures outside the body.

(3) The cultures must be able to reproduce the disease in susceptible animals, and the microbe must be recoverable from the animal in pure culture.

These principles constitute an ideal standard which is not always attainable in practice. Certain microbes that are easy to demonstrate in lesions cannot be cultivated; e.g. the malaria parasite and the *Mycobacterium* of leprosy. Others, like *Treponema pallidum* of syphilis, are so difficult to grow and so readily lose their pathogenicity in culture that it is almost impossible to satisfy the second and third postulates. Finally, there is the gonococcus, which, though easy to find and cultivate, is so finely adapted to the human body that no experimental animal can be found with which to satisfy the third postulate.

Since, however, the full proof has been given in enough diseases to establish the 'germ-theory' as a whole on an unassailable basis, we are justified in other cases in accepting a less rigid standard, especially when it is backed up by circumstantial evidence such as the constant appearance of specific antibodies in the blood.

Pathogenicity. Virulence. Toxins

The chief difference between pathogenic and non-pathogenic bacteria is that the former contain or secrete *toxins*, i.e. substances that damage the tissue-cells. From the biological point of view these substances are of the greatest value to the microbe, since they enable it to survive and multiply in a territory closed to saprophytic bacteria. If saprophytes are introduced by accident or design into the blood or tissues they are quickly removed by the phagocytic leucocytes and endothelial cells, whose function is to ingest and digest foreign particles of all kinds. But when the phagocytes are faced with pathogenic bacteria their functions are in a greater or lesser degree inhibited by the action of the toxins, and the way is thus opened for a further invasion of the body. Owing to the different chemical structure of the cells of different animals, a given microbe is not equally pathogenic to all. Every parasite has its special range of 'hosts', just as each species of animal has its particular set of parasites. Thus a microbe which is pathogenic to man and monkeys may be relatively or quite harmless to rabbits and rats, and so on.

Virulence. This term is used to indicate the variable pathogenic power of different bacterial species or of different races of a single species. All pathogenic species must, by definition, be capable of virulence, but their degree of virulence is by no means a fixed quantity, and they may even produce completely avirulent variants (p. 44). Virulence is estimated by injecting graded quantities of a culture into susceptible animals and determining the least dose that will invariably cause a fatal infection (minimal lethal dose; M.L.D.). Unfortunately both the animals and the microbes have the inherent variability of all living things; and,

moreover, the multiplication that may take place in the animal after injection tends to spoil the quantitative nature of the experiment. Consequently, to obtain accurate results, about 20 animals should be injected with each dose. This, however, is so expensive that in practice we have generally to be content with a much rougher estimate.

Virulence depends on the *toxicity* and the *invasive power* of the microbe—two properties which are partly independent. Some microbes, like *C. diphtheriae* and *Cl. tetani* are very toxic, but uninvasive, while others, like *Myc. leprae*, are invasive but of very low toxicity.

Invasive power. A better term might be 'tendency to dissemination', since, as we have seen (p. 6), invasion is not an active process but a passive distribution of the bacteria by lymph or blood-currents and by wandering phagocytes. It largely depends on the ability of the microbe to resist growth-inhibition and destruction by antibodies and phagocytes (Ch. XV). In many instances this resistance has been shown to be due to the secretion of a protective surface layer—a visible or invisible capsule of gummy toxic polysaccharides (pp. 66, 162 &c).

The extent of the invasion does not depend only on the invasive power of the microbe, but also on the *natural or acquired resistance* of the host (Chap. XV). If the natural defences of the body (p. 227) are in good order, and the microbe is of low virulence, the infection may remain localized. This is what happens, for example, in the trivial skin-pustules caused by *Staphylococcus pyogenes albus*. On the other hand, when a virulent organism such as *Streptococcus pyogenes* is introduced into healthy tissues through some trivial abrasion, it may spread with catastrophic rapidity, and set up a fatal infection of the blood (septicaemia). If the microbe is of very high virulence even the increased

resistance following specific infection or inoculation may not suffice to prevent this calamity.

Toxins, as we have seen, are the constituents or excretions of the bacterial cell on which its poisonous property depends. If the toxin forms an integral part of the cell, liberated only on its death and disintegration, we call it *endotoxin*. If, however, it is a diffusable secretion or excretion of the living cell it is termed *exotoxin*. In most instances the distinction is clear enough, but sometimes it is not easy to decide which type of toxin we are dealing with (atypical toxins). Roughly speaking, if the filtrate of a fluid culture incubated for a few days is toxic to guinea-pigs or rabbits in very small doses it is considered to be an exotoxin; but if such filtrates are comparatively harmless, and if the toxic substance can only be obtained by special manipulations, we call it an endotoxin. The usual procedures for obtaining endotoxins are (1) autodigestion (autolysis) of the cells, which takes place during prolonged incubation in salt-solution; (2) grinding and subsequent extraction of the pulverized cells with water or saline solution.

TABLE V

The best-known toxins

Species	*Type of toxin*
Corynebacterium diphtheriae	Exotoxin
Clostridium tetani	,,
,, *botulinum*	,,
,, *welchii*	,,*
Streptococcus pyogenes	,,*
Staphylococcus aureus	,,*
Bacterium dysenteriae (*Shiga*)	Intermediate
Salmonella typhi	Endotoxin
Vibrio cholerae	,,
Neisseria meningitidis	,,
,, *gonorrhoeae*	,,

* Atypical exotoxins, with a high heat-resistance.

Properties of toxins. All toxins are inactivated by *heat*, but of the two types the exotoxins are much the more sensitive, losing their toxicity in 30 to 120 minutes at 60° C. or less: whereas the endotoxins can generally stand an hour at 80° C. without loss of strength.

Typical exotoxins, when injected into animals in subtoxic doses, readily give rise to powerful neutralizing antibodies or *antitoxins*. On the contrary, the antibody-producing power of endotoxins is generally very feeble.

Measurement of the strength of toxins. We have seen that in the estimation of the killing power of a culture the inevitable variation of sensitiveness of the individual animals employed makes accurate results impossible with small numbers of animals. This is equally true of toxin-measurements. For an exact estimation of a toxin it is necessary first to find the approximate strength by injecting graduated doses into one or two animals, and then to test a range of finely graded dilutions on either side of the estimated value by injecting each dilution subcutaneously or intraperitoneally into some twenty animals. The dose that kills half of the animals (the next larger dose killing a higher, the next smaller a lower proportion), may be taken as the minimal lethal dose (M.L.D.). This method, however, being too expensive for common practice, is usually replaced by the method of *intracutaneous injection* of minute graded doses into guinea-pigs or rabbits. Six, eight, or more dilutions are injected into different skin-areas of one animal; and the least quantity that produces a local inflammation, i.e. redness and swelling at the point of injection, is taken as the minimal toxic dose.

A third method, the *flocculation-reaction*, gives an indirect estimate of the strength of a toxin by estimating its precipitating power against an antitoxic serum

of known potency (p. 132). The toxin is added in graded dilutions to a constant volume of the antitoxin, and a note is made of the dilution that gives the quickest precipitation or flocculation. This method, though very useful, is not quite accurate, since the combining power of a toxin does not run completely parallel with its toxicity.

Toxoid. When stored for some time, exotoxins lose much of their toxicity; but, curiously enough, they retain both their power of combination with antitoxin (e.g. as shown by the flocculation test) and also their ability to cause antitoxin-production in animals. A similar, but more complete, change can be effected either with moderate heat or by treatment with formalin. The resulting atoxic but antigenic substance (toxoid; formol-toxoid) has proved useful for the immunization of animals and human beings (pp. 136, 170).

Haemolysis. The toxins of the following bacteria have the property of dissolving erythrocytes: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Clostridium tetani*, *Clost. welchii*; and some of the pathogenic varieties of *Bacterium coli*. Since the excretions of certain nonpathogenic bacteria have the same property (e.g. *Bac. megatherium*, *Vib. El Tor*) haemolysis is not necessarily a sign of virulence.

Tests for haemolysis. (1) Cultivation on a blood-agar surface. A translucent zone develops round the colonies. (2) Growth in broth for 18–24 hours; addition of some washed erythrocytes; incubation for an hour or so. The released haemoglobin diffuses through the fluid. Of the two tests the second is the more reliable.

Leucocidal action. Most toxins injure or kill leucocytes—clearly a very important action. It is supposed to reside in special toxin-components, *Leucocidins*; but since they have not been isolated, their separate existence is doubtful.

Variation of virulence. Bacteria generally undergo a loss of virulence during prolonged cultivation; and this is accompanied by an antigenic reduction and the appearance of 'rough' variants (p. 43).

If a culture undergoing this change is analysed by testing the virulence of a number of substrains raised from single cells, it is often found that the loss is unevenly distributed; one substrain may be fully virulent, another almost harmless. By passing a culture in this mixed condition through a susceptible animal it is sometimes possible to restore it to full virulence, since only the pathogenically 'fit' substrains can resist the self-purifying action of the tissues. The modification of a racial character by natural selection is here occurring under our very eyes.

The opposite effect, *attenuation* of virulence, can be obtained by treatment of the bacteria, with moderate heat or low concentrations of antiseptics, insufficient to kill, but enough to weaken the cell. It was with vaccines (p. 248) of *Bacillus anthracis*, attenuated by prolonged cultivation at 40°–43°C., that Pasteur carried out his pioneer experiments on the protective inoculation of sheep and oxen against anthrax (p. 248).

The attenuation that occurs during prolonged cultivation on unfavourable media has been used in France and elsewhere for the preparation of a living, avirulent, protective vaccine against tuberculosis—the famous B.C.G. (p. 153).

CHAPTER V

THE PYOGENIC COCCI: STAPHYLOCOCCUS, STREPTOCOCCUS, AND NEISSERIA

STAPHYLOCOCCUS (Micrococcus)

(*Suppuration; furunculosis; osteomyelitis, &c.*)

THIS genus consists of Gram-positive, non-sporing, unencapsulated, aerobic cocci, growing in grape-like

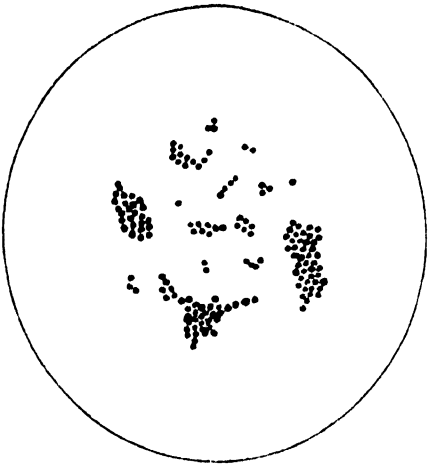

FIG. 7. *Staphylococcus pyogenes*

clusters (Table VI and Fig. 7). The constituent species are difficult to classify owing to their lack of sharply differential characters, but we may provisionally distinguish between the pyogenic (pus-forming) *Staph. pyogenes*, with its golden and white varieties, the colourless commensal of the skin, *Staph. epidermidis albus*, and a lemon-yellow saprophyte, *Staph. citreus*.

TABLE VI

SPECIES	MORPHOLOGY: *Form*	*Motility*	*Spores*	*Capsules*	STAINING: *Gram*	*Acid fast.*	BIOCHEMICAL: *Oxygen*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Staphylococcus pyogenes	Clumped spheroid	0	0	0	+	0	AER	+	AC	Ordinary media
Streptococcus pyogenes	Chained spheroid	0	0	0		0	AER	0	A	Do. (likes serum at first)
Streptococcus pneumoniae (pneumococcus)	Lanceolate diplococcus; short chains	0	0	+		0	AER	0	A(C)	Like *Str. pyog.*
Sarcina (lutea, &c.)	Spheroids in packets	0	0	0	+	0	AER	0	0	Ordinary media
Micrococcus tetragenus	Spheroids in tetrads	0	0	0	+	0	AER	0	?	Do.
Neisseria gonorrhoeae	Semilunar diplococci & clumps	0	0	0	0	0	AER	0	0	Needs serum, &c., in media
Neisseria meningitidis	Do., do.	0	0	0	0	0	AER	0	0	Needs some enrichment at first

AER = Aerobic; A = Acid; C = Clot; ( ) = Variable; 0 = Negative; + = Positive; ? = Uncertain.

Staphylococcus pyogenes

(varieties: *aureus*; *albus*)

This organism is generally met with in the pus and tissues in acute suppuration, but it may also be found on healthy cutaneous or mucous surfaces.

Growth on agar rapidly produces a dense paint-like film, coloured deep or pale golden-yellow (*aureus*), or white (*albus*). The colonies are rather large, circular, and opaque. A zone of haemolysis may be seen around colonies on blood-agar.

In fluid media it produces turbidity and deposit. It has strong powers of growth and survival, and it needs an hour of moist heat at 60° C. for sterilization. It has been shown by agglutination and absorption tests to be antigenically distinct from *Staph. epidermidis albus*.

The *pigment* is a lipochrome soluble in alcohol, ether, &c. Not only does the tint differ in the different races but its intensity varies in the same race under different conditions. A culture, for example, may appear white after 24 hours' growth, but develop yellow pigment on further incubation.

Pathogenicity. Toxins. The species is *naturally pathogenic* to most warm-blooded animals, and experimentally to rabbits, mice, &c., causing local suppuration and often general abscess-formation (pyaemia). Its virulence, which is highest in the *aureus* variety, is due to *both exo- and endotoxins*. The latter is both haemolytic and leucocidal; and when perfused through an animal's lung it liberates *histamin*, to which some of its toxic effects are probably due. Temporary capsules have recently been demonstrated *in vivo* in virulent strains (p. 48).

Staphylococcal infection in man. The typical lesion is an acute suppurative local inflammation, consisting

of a central necrosis (the core) surrounded by a zone of mainly polymorphonuclear leucocytes which merges into a layer of granulation tissue as the centre liquefies and the focus becomes an abscess. The regional lymph-nodes often become infected, especially in children. The infection does not usually spread farther, but it occasionally reaches the blood by the agency of a 'septic' clot in a vein contiguous to the primary focus; and serious or fatal 'pyaemic' abscesses may thus develop in various parts of the body. Pustular skin-eruptions, boils, carbuncles, and septic sores are caused by the multiplication of the coccus in sweat-ducts, hair-follicles, and minor wounds. The escape of a few cocci into the blood from some trivial lesion may give rise to a focus of suppuration in a bone (acute osteomyelitis) or in some other organ. In the severer lesions the cocci are practically always of the *aureus* type; while in chronic benign pustules like those of acne they are generally white.

Occasional outbreaks of food-poisoning are caused by the growth of staphylococci in moist food.

Diagnosis is straightforward, since the clumps of Gram-positive cocci in dried films of polymorphonuclear pus are usually easy to recognize; and cultivation by smearing a loopful of the material on an agar slope presents no difficulty.

Immunity and therapy. The natural resistance of man to this ubiquitous parasite is evidently high, or it would long ago have destroyed our race. But recovery from infection does not confer complete or lasting immunity from further attacks.

Attempts to induce passive immunity (p. 249) by means of antiserums have uniformly failed, but therapeutic active immunization (vaccine-therapy) with killed suspensions has met with considerable, if irregular, *clinical* success. The word clinical needs to

be emphasized because the conditions of human life make it practically impossible to estimate scientifically the results of any form of vaccine-therapy. Yet there is no doubt that a complete cure from recurrent boils may follow a dose or two of autogenous vaccine. Toxoid (formolized toxin, p. 51) has shown promise as a therapeutic stimulant of antitoxic immunity.

Staphylococcus epidermidis (albus)

A group of non-pathogenic, non-haemolytic, white cocci of the skin, especially of the scalp. By the ordinary tests they cannot be distinguished from the white variant of *Staph. pyogenes*; but they differ in their lack of haemolysin, in their complete avirulence for animals, and in failing to react with agglutinating or precipitating antisera made with suspensions or extracts of the pathogenic species.

Micrococcus tetragenus (Table VI)

The chief characteristic of this coccus is its arrangement in tetrads; i.e. groups of four cells arranged symmetrically. Only a proportion of the elements are so arranged, pairs and larger groups being also common. Its cultural characters are much like those of the white staphylococci. It is a normal inhabitant of the body (Table XXVI, p. 266) and is usually harmless, but it occasionally causes suppuration and even septicaemia. Numerous similar species, often giving pigmented growth, occur in air, water, and elsewhere.

Sarcina (Table VI)

This is a group of yellow cocci very like *Micrococcus*, but growing typically in cubical packets like roped bales of goods. They occur in air, water, and in the stomach and intestines of man and animals. Though normally non-pathogenic, they are sometimes found in the pus in suppurative conditions.

STREPTOCOCCUS

(Throat infections; scarlet fever; endocarditis; suppuration; septicaemia; erysipelas; pneumonia, &c.)

This genus consists of chained, non-motile, non-sporing, Gram-positive and, with a few exceptions, aerobic cocci. The innumerable races are divisible according to their cultural and biochemical characters and parasitic habits into four groups, of which the first two are by far the most important: (1) *Streptococcus pyogenes*; (2) *Streptococcus pneumoniae* (pneumococcus): (3) The mouth-streptococci, *Str. viridans* and *Str. saprophyticus*; (4) The 'faecalis-lactis group' comprising the harmless *Str. faecalis* or enterococcus, and the milk-souring *Str. lactis*.

Streptococcus pyogenes

(Syn. Str. haemolyticus)

This organism is the cause of a great variety of suppurative inflammations in all parts of the body, especially the throat, tonsils, skin, and subcutaneous tissues. Specially toxic varieties cause scarlet fever. A very dangerous blood-infection (septicaemia) may result from any of these conditions, and also from infection of the uterus after child-birth (puerperal fever).

The general characters of the organism are shown in Tables VI and VII and Fig. 8. It usually grows in chains of between ten and fifty cells of about 0·75 μ diameter, but shorter chains and pairs (diplococci) are often to be seen.

Culturally, it is rather delicate and requires frequent subcultivation. The growth on *agar* is not profuse unless the medium is enriched with blood or serum. In *broth* the growth is granular and collects on the

walls and bottom of the tube. The *colonies* on solid media are small, moderately opaque, and unpigmented. On blood-agar in 24 hours a zone of complete haemolysis (called β-haemolysis) is seen round the colonies. *Str. pneumoniae* and *viridans* on the contrary produce at first an opaque greenish ring which, on prolonged

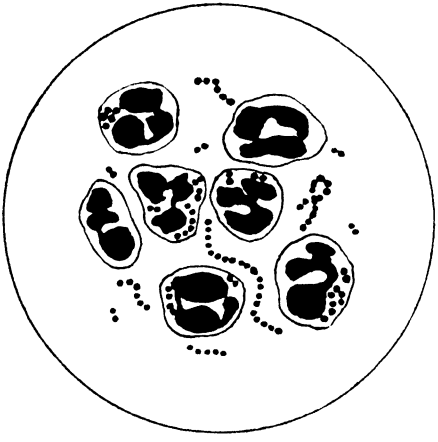

FIG. 8. *Streptococcus pyogenes* in pus

incubation, may be surrounded by a zone of weak (α-) haemolysis.

Biochemical reactions. Several carbohydrates are fermented, without gas-production. Generally speaking, the failure of the organism to ferment *mannitol* distinguishes it from *Str. faecalis*; and a similar absence of effect on *inulin* separates it from *Str. pneumoniae*; but these reactions are hardly constant enough to be relied upon for differentiation. *The antigenic structure* of the group is very complex and incompletely understood. In addition to some 30 'types', each with

TABLE VII. *Differential Characters of Streptococci*

Species	*Morphology in fluid media*	*Haemolysis on blood-agar*	*Exotoxin*	*Abnormally high heat-resistance*	*Solubility in bile-salt solutions*	*Type of disease in man*	*Pathogenicity to mice*	*Milk*
Str. pyogenes	Long or short chains	Rapid, strong, β lysis	+	0	0	Acute suppuration, septicaemia, &c.	+	Acid only
Str. pneumoniae	Lanceolate diplococci and short chains capsulated	Green halo, slow α lysis	0	0	+	Lobar pneumonia; infl. of serous membranes	+	Acid and often clot
Str. viridans	Long or short chains	Green halo, slow α lysis	0	0	0	Chronic endocarditis	0	Acid and often clot
Str. saprophyticus	Long or short chains	0	0	0	0	0	0	Acid and often clot
Str. faecalis (enterococcus)	Diplococcus and short chains	No change; (rare β lysis)	0	+	0	None	0	Acid and clot

its type-specific antigen, certain sub-groups are antigenically distinguishable by precipitin-reactions with bacterial extracts. One of these, 'Group A of Lancefield', is of primary importance as containing the great majority of the human pathogenic types.

Pathogenic action. The organism is naturally pathogenic to man and domestic animals. Of the laboratory animals the mouse is by far the most susceptible to experimental injection (Table VII).

The lesion in man in all its forms is an acute inflammation with aggregation of polymorphonuclear leucocytes, oedema, and suppuration; sometimes localized in abcesses, but often diffuse and spreading, as in erysipelas and cellulitis. The strong invasive tendency of the organism only too often leads to septicaemia. It produces an exotoxin, the potency of which varies greatly in different races. The more toxigenic cause erythematous rashes, of which scarlet fever provides the most typical example. In this disease the organism can practically always be found in the throat, and the infection can be reproduced in man by spraying pure cultures into the throat. Even filtered cultures, free from bacteria but containing toxin, if injected into a human subject cause a transitory fever and rash indistinguishable from a mild attack of scarlet fever. Throat infections with *Str. pyogenes* are often followed, after an interval, by *rheumatic fever*; but since streptococci are not demonstrated in the blood or joints, their connexion with the disease is uncertain.

Toxins. Str. pyogenes produces both exo- and endotoxin. The former is present in filtrates of young cultures and contains at least two components: one haemolytic and leucocidal, destructible at 60° C.; the other erythrogenic or rash-producing, and needing 90° C. or over for inactivation. The rash is probably due to the liberation of a histamin-like substance by

cells damaged by the toxin. The production of a rash depends solely on the (variable) potency of the erythrogenic factor; but the *invasive power* of the cocci depends both on the leucocidal power of the exotoxin and on the antiphagocytic substance in the surface layer (temporary capsule). The exotoxin also contains a *fibrinolysin*, which assists invasion by preventing the coagulation of plasma and the consequent blocking of capillaries round a focus of infection. Scarlatiniform rashes occur not only in the highly toxigenic throat-infections which we class as scarlet fever, but also in infections of the skin, connective tissues, uterus, &c. ('septic rashes'; 'surgical scarlet fever', &c.). Proof that the rash of scarlet fever is due to the toxin is given by the *Schultz-Charlton* reaction, in which a minute quantity of specific *antitoxin* injected intradermally causes a local blanching of the rash. This has been used in diagnosis, but is not reliable. The production of a rash, as also of other symptoms of intoxication, depends not only on the toxicity of the coccus but equally on the *susceptibility* of the infected subject to the toxin. Thus the same streptococcus may cause 'scarlet fever' in one person, a rashless tonsillitis in another, and a 'septic finger' in a third.

The Dick test enables us to estimate with moderate accuracy the sensitiveness or resistance of a person's tissues to the *erythrogenic fraction* of the toxin. It is based on precisely the same principles as the Schick test for susceptibility to diphtheria. A minute quantity of toxin injected intracutaneously produces a local pink swelling (i.e. a positive reaction) in individuals whose blood is devoid of natural or acquired antitoxin, but no reaction in those who have acquired some immunity by previous infection or sub-infection (p. 9). The test is no good in infants, whose skin is insensitive to the toxin and who therefore all give negative re-

actions although generally susceptible to the disease. Otherwise the effects of age, conditions of life, &c., on the reaction are similar to their effects on the Schick test (*q.v.* p. 133). The Dick reaction does not completely decide whether an individual is immune against scarlet fever, since factors other than sensitiveness to the erythrogenic toxin also come in; but there is a high statistical correlation between Dick-negativeness and immunity.

Spread of infection. In scarlet fever, tonsillitis, &c., infection is spread chiefly by droplets from the throats of patients and carriers. In pre-Listerian days, when puerperal fever decimated parturient women, the streptococcus was carried from case to case on the unwashed hands of doctor or midwife. Nowadays, it appears that the patient's or attendant's throats are often to blame, and that simple gauze masks help to prevent this still formidable disease.

Among other vehicles of streptococcal infection are pus and scabs from wounds and sores, epidermal scales after toxic rashes and milk contaminated by human carriers in farm or dairy.

Diagnosis. Bacteriological proof of scarlet fever is a complicated matter involving the identification of the streptococcus cultivated from the throat as one of the 17 antigenic types that cause scarlatinal rashes. Special laboratory facilities are therefore necessary. The mere presence of *Str. pyogenes* is not enough, as it may be found in non-scarlatinal sore-throat, in tonsillitis, and in healthy carriers. In *inflammatory and suppurative* conditions the diagnosis is made by direct microscopic examination of dried films of pus or exudates, supplemented by cultivation on blood-agar to demonstrate haemolysis. For *septicaemia* cultivation of the blood in broth is necessary, since the cocci can seldom be found by direct examination.

Immunization and therapy. Antitoxic serum is made from the blood of horses subjected to a course of injections of the streptococcal exotoxin. Experiments on animals and clinical trials on man have shown that the antibodies it contains neutralize the exotoxin and prevent its deleterious actions on the tissues; but its effect on the invasive properties of the microbe are less striking. Anti-endotoxic serums prepared by the injection of the endotoxins of the organism into large animals have proved experimentally effective, but only against the particular race of streptococcus concerned, because of the diversity of the endotoxic antigens of the group.

Active immunization against scarlet fever with small injections of toxin is done in the same way as for diphtheria (p. 136). Toxoid, which is difficult to prepare without loss of antigenicity, is now being tried in various quarters. Recent experience has, however, thrown doubt upon the utility of this purely antitoxic immunization, which, though it abolishes the rash, leaves the chief menace of the disease, streptococcal invasion, untouched.

Chemotherapy of streptococcal infections, see p. 259.

Streptococcus pneumoniae

(Syn. Pneumococcus)

This organism is mainly found in the sputum and lung-lesions of lobar pneumonia and in the resulting pleural exudates. Also in acute and chronic catarrhal inflammation of the air-passages, usually in conjunction with other bacteria; in the purulent fluids of pneumococcal peritonitis, meningitis, arthritis, pericarditis, and pleurisy, and finally in the throats of a variable number of healthy persons.

Its main characters are sufficiently shown on pages

54, 60, and in Fig. 9. The lanceolate elongation of the cells in the long axis of the pairs is characteristic, though shared to a certain degree by the coarser *Str. faecalis*. Capsules are often difficult to demonstrate in cultures, but are always formed in the tissues.

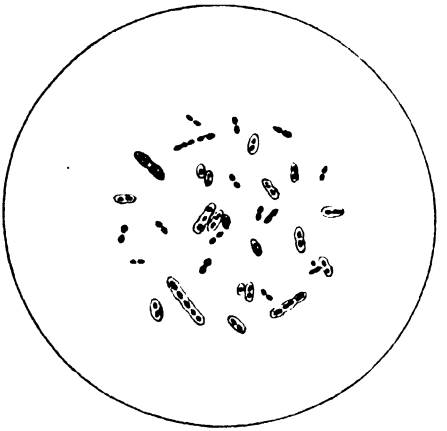

FIG. 9. *Streptococcus pneumoniae* (with and without capsules)

For first isolation agar enriched with serum or blood, fresh or heated, is advisable. The organism grows thinly on plain agar, and rather better in broth, producing a uniform turbidity. It acidifies without gas-production several carbohydrates, including *inulin*, which is not fermented by the other species of the genus. *Solubility in bile or solutions of bile-salts* is a curious and unique character of the species.

The pneumococcus is highly *virulent* for mice and guinea-pigs, but much less so for larger animals.

The lesion in man is of the acutely inflammatory and suppurative type, the polymorphonuclear leucocyte being the preponderant cell. In pneumonic and serous infections there is a coincident pneumoccoccal septicaemia, and it is on the severity and duration of this factor that the danger of lobar pneumonia largely depends. The phagocytes are unable to cope with the rapidly multiplying cocci until specific antibodies begin to appear in the blood on the 4th to the 7th day of the illness, when, in favourable cases, a crisis of bacterial destruction and resolution of the inflammatory exudate takes place.

Toxins and antigens. The pneumococcus produces no exotoxin. Its invasive powers are due to its antiphagocytic capsular antigens. Antigenic analysis with antiserums made with a variety of races has shown that the species is composed of at least 32 serological types of which the first three are especially common in pneumonia. Types I and II are identified in practice by agglutination with type-specific serums, and type III is identifiable without agglutination by its property of producing a moist, mucoid growth on solid media. The remaining types used to be classed as Group IV (or type IV), but are now given separate numbers.

Chemical analysis has established the important fact that the antigenic individuality of the types is due to different polysaccharide components in the capsule. Isolated in the pure state, these substances react by specific precipitation with the appropriate antiserum, though, curiously enough, they are not in themselves antigenic, i.e. they are incapable of stimulating antibody formation. It is only in their natural state of combination with the non-specific protein-components of the coccus that they can act as antigens. To distinguish these substances from true antigens and to emphasize their combining properties, the name

Haptens has been given to them. Other bacteria, perhaps all, possess similar components (p. 238).

Variation of the coccus from the original smooth phase to the rough involves the disappearance of the specific polysaccharide and a consequent loss of virulence; and in this connexion a very interesting observation has been made. If a living rough culture is mixed with a killed smooth suspension *of another type*, and injected into a mouse, the animal dies and its blood is found to contain smooth pneumococci *of the type of the killed suspension*. The rough living coccus has thus been stimulated by the alien specific smooth substance to produce the same substance for itself. A similar transformation has lately been effected by the simple cultivation of a rough strain in medium containing killed pneumococci of another type.

Immunization and therapy. In order to supply specific antibodies as soon as possible in pneumonia, antiserums are made by injecting horses with killed and later living pneumococci. Serums for types I, II, and III have been considerably used, and progress is being made with other types. The organism from the patient is typed by agglutination with specific serums, or by microscopical observation of the swelling of the capsule under the influence of the specific serum.

Clinical records show a statistically significant reduction of case-fatality in type I pneumonia, but the effect of the other type-serums is not yet clear.

Active immunization of limited communities against the most prevalent types of pneumococcus, by means of killed suspensions (vaccines), met with apparent temporary success in reducing the incidence and severity of the disease in the S. African mines; but here again the diversity of the antigens and the fluctuations of the infecting types have proved serious obstacles.

The recent discovery that the enzymes of a certain saprophytic bacterium dissolve the capsules of type III pneumococcus, and thus protect mice experimentally against it, may have an interesting future.

The Mouth-Streptococci

Streptococcus viridans. This name originates from the greenish ring (p. 59) round the colonies on fresh or heated blood agar, apparently due to the formation of H_2O_2. In this it resembles *Str. pneumoniae*, but differs in other characters, Table VII, p. 60. The individual races show much variation in biochemical action and antigenic structure. These cocci are fundamentally normal commensals of the mouth and tonsillar crypts. They are also regularly found at the roots of dead or diseased teeth, whence they may pass into the blood. There they are almost always disposed of harmlessly, unless they settle on a damaged heart-valve, where they may set up a *subacute infective endocarditis* or *endocarditis lenta* which is generally fatal. They have also occasionally been found in the joint-fluid in chronic rheumatism, but their causative role is unproven.

Streptococcus saprophyticus. This is a convenient name for cocci which give neither α nor β haemolysis. They are sometimes called the γ-type, and are like *Str. viridans* in other cultural characters, but so far as is known are completely non-pathogenic.

Streptococcus faecalis

(Enterococcus)

A normal inhabitant of the digestive tract, especially in its lower half. Usually harmless, it appears to be sometimes capable of causing inflammatory irritation in the urinary system, and may be found in association with other bacteria in the peritoneal effusion of perforative

appendicitis. Only a few of the many races tested have shown even the mildest pathogenicity for animals (rabbits). The toleration of relatively high temperatures, such as 60° C. for 30 min., is a peculiar characteristic of the species (Table VII), as also is its power of fermenting *mannitol*. It is much more robust than the other streptococci and grows readily on gelatin at 22° C., sometimes causing liquefaction. The common milk-souring *Str. lactis* is of the same general type.

NEISSERIA

(*Gonorrhoea; meningitis; catarrh*)

This genus consists of Gram-negative, non-motile, non-sporing, unencapsulated, aerobic cocci, growing in double-hemispherical pairs and clumps; mostly delicate and needing special culture-media. It includes the important *N. gonorrhoeae* and *N. meningitidis*, the feebly pathogenic *N. catarrhalis*, and a number of harmless nasopharyngeal varieties classed together as *N. pharyngis*.

Neisseria gonorrhoeae

(Gonococcus. Neisser 1879)

This species, whose main characters are given on p. 54, is most often found in the pus from acute and chronic inflammation of the human urethra (Fig. 10). It also causes acute conjunctivitis and (more rarely) inflammation of the mouth and rectum. The characteristic diplococcal form is best seen in pus, where the cocci are mostly in pairs like two half-moons with their flat sides contiguous. In cultures one sees chiefly spherical or oval cocci (about 0·6 to 1·0 μ in diameter) in clumps, though typical diplococci can usually be found.

Cultivation is not easy, and the addition of serum or blood to agar is essential both for isolation and

maintenance. At temperatures below 30° C. no growth takes place. Unless kept in a closed, moist jar at 37° C. cultures tend to die out in a few days, but under the best conditions they will often survive for a month.

The colonies, which take 48 hours to grow to full size, are small, round, translucent, smooth, viscid, and

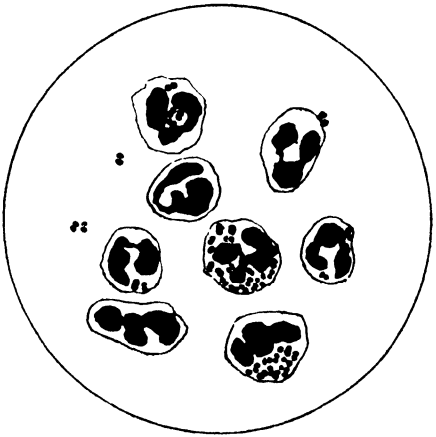

FIG. 10. Gonococci in gonorrhoeal pus

greyish-white. In long-cultivated races rough colonies are sometimes seen.

Growth in *serum-broth* is scanty, with slight turbidity and some deposit. *Resistance* to desiccation and antiseptics is feeble, and since the organism cannot long survive outside the body the statements of patients that they have contracted gonorrhoea from lavatory seats should be taken with reserve.

Biochemical actions are mainly negative. Indole is not produced, and glucose is the only carbohydrate fermented (without gas-formation).

The antigenic characters of the species are not well known. By means of agglutinating serums obtained by injecting animals with various races it is divisible into several rather indistinct groups, in one of which about three-quarters of the urethral strains are found. There is a considerable antigenic relationship between the gonococci and the meningococci.

The serum of injected animals contains complement-fixing and opsonic antibodies, but we have no certain means of demonstrating any protective quality. The gonococcus is not truly *pathogenic* to any creature other than man, but when it is injected in large doses, whether alive or dead, into the peritoneum of mice, rabbits, or guinea-pigs, its *endotoxins* cause peritonitis and death. No multiplication takes place, and the tissues at death may be sterile. There is no evidence of the production of an exotoxin.

The lesion in gonorrhoea is an acute suppurative inflammation. The exudate as a rule shows extensive phagocytosis of the cocci by polymorphonuclear leucocytes. The urethral mucous membrane becomes ulcerated, and extension to the prostate gland is frequent. As complications we find cystitis, orchitis, epididymitis, and, in women, endometritis, salpingitis, and peritonitis. An intermittent invasion of the blood, which rarely develops into a fatal septicaemia or endocarditis, is more often signalized by acute arthritis or 'gonorrhoeal rheumatism', in which the coccus may often be isolated in pure culture from the synovial exudate.

Conjunctivitis is most common in the form of 'ophthalmia neonatorum', which is contracted at birth during passage through the infected maternal genitalia.

Channels of infection. The gonococcus is usually implanted directly from mucous membrane to mucous membrane during sexual intercourse. After recovery from an acute attack the patient may remain a chronic

carrier with *obstinate deep lesions not causing severe symptoms nor interfering with his sexual activities.* It appears also that the carrier-state can arise without any symptoms of an attack, so that the transmission *of infection by a person who has never 'had gonorrhoea' is by no means an impossibility.*

Diagnosis is made by the examination of dried films of pus stained by Gram's method. The characteristic Gram-negative, intracellular diplococci indicate an infection of the *Neisseria* group, and a knowledge of the source of the pus usually excludes *N. meningitidis* or *catarrhalis*. Cultivation on serum-agar should be done in difficult or important cases, but negative results must be interpreted with reserve.

In the later stages of acute infection, in chronic gonorrhoea and arthritis the blood-serum usually contains antibodies which can be demonstrated by the *complement-fixation* test (p. 180), a suspension of several strains of the organism being used as 'antigen'. Though the reaction is positive in only about 75 per cent. of cases, it is a useful aid to a diagnosis which may otherwise be extremely difficult.

Immunity and immunization. Acute gonorrhoea does not confer long-lasting immunity; nor have we any proved means of producing either active or passive immunity by injections of vaccines (i.e. killed or living suspensions of the coccus) or of antiserum. *Chemotherapy* with sulphanilamide, &c., has given promising results (p. 259).

Neisseria meningitidis (Meningococcus)

(*Cerebrospinal meningitis*)

In its main characters (Table VI, p. 54) this organism resembles *N. gonorrhoeae*. We need only describe the points of difference. The meningococcus

occurs in the purulent cerebrospinal fluid in cases of epidemic and sporadic cerebrospinal meningitis; in the nasopharynx of those who have been in association with cases of the disease (contact-carriers); and in a varying proportion of the general population (carriers).

Culturally it is less exacting than the gonococcus; it grows more profusely and forms larger colonies. Although most long-cultivated races will grow feebly on plain agar, and rather better in broth, for first isolation the organism needs accessory growth-promoting factors such as are found in serum, ascitic fluid, eggs, and pea-flour. For general purposes coagulated egg is the most suitable medium; growth on it is rapid and profuse, and the antigenic qualities and virulence of the organism are well maintained. Swelling and disintegration (*autolysis*) of the cells is common in cultures; and to be sure of a successful subculture a considerable mass of growth must be transplanted.

Biochemical actions are like those of the gonococcus except that *N. meningitidis* acidifies both glucose and maltose.

Antigenic character and variation of the coccus are of importance in the epidemiology, diagnosis, and treatment of the disease. Agglutination and absorption tests distinguish two main groups of varieties. Group I strains contain two main antigens, present in varying proportions. All therefore cross-agglutinate, though to different degrees. Group II consists of miscellaneous antigenic types which cross-agglutinate very little. Variable slight cross-agglutination may also be seen between the groups, owing to a basic group-antigen. The serological identification of Group I strains is fairly easy; that of Group II, owing to its heterogeneity, is more difficult. In epidemics a single race is usually responsible throughout, and it is most

commonly of Group I. Cocci of Group II are prevalent in sporadic cases and carriers.

The *toxic action* of the organism, like that of the gonococcus, is due to *endotoxin*, which appears to be neither specific nor antigenic. But from whole cocci specific *polysaccharide haptens* can be extracted. A recent claim to have prepared meningococcal exotoxin (and antitoxin) has not been confirmed.

Cerebrospinal meningitis and its sporadic form, post-basic meningitis, occur naturally only in man, but can be experimentally induced in monkeys by intrathecal injection of pure cultures. The cerebral and spinal meninges undergo acute suppurative inflammation and the cerebrospinal fluid becomes turbid with leucocytes and cocci. Though a very formidable disease with a 50 per cent. case-mortality, it is luckily not very common, nor does it often cause large epidemics. Our natural resistance to the coccus is evidently high.

On arrival in the nasopharynx, to which it has been conveyed in a droplet of mucus expelled by some patient or carrier, the meningococcus multiplies slowly and gives rise either to no local symptoms or to a mild catarrh. If the microbe is of high virulence or the person of low resistance cerebral invasion occurs. But many races of meningococci are relatively avirulent and only effect a temporary commensal colonization.

We do not know for certain by what route the coccus reaches the central nervous system. There are two possibilities: (1) Direct, through lymph channels along the olfactory nerve-fibres which pass through the cribriform plate. (2) Indirect, through the blood-stream. Cultivation from the blood is successful only in about one case in four, but slight, transient blood-infection may be far commoner than these results suggest. Against the nasal route we have the fact that

so far as can be experimentally ascertained by means of intradural injection of dyes in animals the lymph-flow in that region is nosewards.

Invasion of tissues other than the meninges is rare, but endocarditis, arthritis, and abscess of the kidney are the occasional results of dissemination by the blood-stream.

Spread of infection. The sole source and reservoir of infection is man. It was observed during the Great War that in the period before an outbreak in a given battalion the microbe spread progressively amongst the men, and when the percentage of carriers reached 20 or 30, a crop of meningeal infections tended to break out. But it has since been proved that much higher carrier-rates can occur without any outbreak, and that outbreaks happen without a high carrier-rate. It is most likely that differences both in virulence of the prevalent strain and of hygienic and nutritional fitness of populations play a determining role. Overcrowding is also important. It has been shown that during sleep the effective 'spraying distance' for droplet infection is only about 3 feet, and that the carrier-rate depends greatly on the spacing of the beds, thus:

Distance between beds.	*Percentage of carriers*
Less than 9 in.	30 or more
1 ft.	20
1 ft. 4 in.	9 to 10
2 ft. 6 in.	5
3 ft.	2 or less

There is good evidence that the recurrence of epidemics in certain institutions has been prevented by the timely introduction of wider bed-spacing.

The *diagnosis* of meningococcal meningitis is made by microscopic examination of Gram-stained films of

the purulent fluid obtained by lumbar puncture. The paired semi-lunar Gram-negative cocci, mostly in phagocytes, are only distinguishable from gonococci by a knowledge of the source of the material. Cultures for confirmation of the diagnosis and for biochemical and agglutinative identification are made by smearing some of the purulent fluid on the surface of egg-medium or boiled blood-agar. *The precipitin reaction,* in which a specific antiserum is used to detect soluble haptens in centrifugalized c.s. fluid from the patient, is practically always positive in c.s. meningitis and very seldom in other conditions.

To detect carriers the nasopharynx is swabbed with a bent swab which is then rubbed on the surface of the medium in slope-tubes or Petri-dishes. Suspicious colonies are examined microscopically and subcultivated for serological identification.

Serum-therapy. By treating horses to a long course of weekly injections first of killed, then of living, meningococci an antibacterial serum specific for the type or group injected can be produced. Such a serum, when injected into small animals with an otherwise lethal dose of living cocci or their endotoxin, protects them from the toxic effects. A very rough idea of its potency can thus be obtained, but accurate estimation is not at present possible, owing to our inability to standardize the endotoxin.

Statistical proof of the value of serum-therapy is not available owing to wide variations in the methods of preparation of the serum, in the strains used, and in the doses given; also to the impossibility of arranging clinical treatment in the form of a scientific experiment with adequate untreated controls. But the collected figures strongly suggest that treatment with a good brand of serum, especially in Group I infections, substantially reduces the case-mortality of the disease.

The serum is usually given intrathecally, but recent work suggests that the intravenous route may be better.

Chemotherapy, see p. 259.

Other Species of Neisseria

Amongst a number of more or less harmless Gram-negative cocci found in the nasopharynx, *N. catarrhalis* stands out as a potential cause of chronic catarrhal inflammation, though the evidence of its pathogenicity is only circumstantial. It is undoubtedly more prevalent and profuse on unhealthy than on healthy mucous membranes.

The following characters distinguish it from *N. meningitidis* (or *N. gonorrhoeae*): (1) Ability to grow on plain agar, even at 20°-25° C. (2) Larger, denser, and whiter colonies on egg, &c.; not readily emulsifiable in saline solution. (3) Lack of fermenting power for glucose, maltose, &c. (4) Negative agglutination reaction with meningococcal serums. Although it has no power of setting up infection in animals, large doses of living or dead culture have a toxic effect on rabbits and guinea-pigs.

Neisseria pharyngis (Micrococcus flavus) is the name given to a group of non-pathogenic nasopharyngeal cocci which resemble *N. meningitidis* morphologically, but are generally distinguishable in culture by the yellowness of the growth or by the dry toughness of the colonies. A lack of toxicity to animals and the ability to grow at 20° to 30° C. are additional points of distinction. The agglutination reactions with meningococcal type-serums are negative, but this is not conclusive, and since some races strongly resemble the meningococcus in colour, growth-requirements, and other properties, the differentiation may be almost impossible.

CHAPTER VI

BACTERIUM

(*Enteric fever; gastro-enteritis; bacillary dysentery, &c.*

THIS large genus of smallish, Gram-negative, non-sporing, freely growing rod-shaped intestinal bacteria comprises: (1) The pathogenic, motile *Salmonella* group (after Salmon, the discoverer of the first species of this type). The most important species in this group are *S. typhi* (Eberth. 1880), *S. paratyphi A, B, and C* and the food-poisoning organisms *S. enteritidis* (*Gaertner*) and *S. typhimurium* (syn. Bact. aertrycke). (2) The non-motile dysentery group, *Bacterium dysenteriae* (*Shiga; Flexner; Sonne &c.*). (3) A miscellaneous group of occasionally pathogenic commensals of the intestine or air-passages, including *Bact. coli*, the capsulated *Bact. friedländeri*, and *Bact. faecalis alkaligenes*.

The *morphological* and *cultural* characters of the various species in the genus are so much alike that their differentiation depends largely on the subtler biochemical and antigenic reactions.

Biochemical action. The gassy fermentation of *lactose* distinguishes *Bact. coli* and other related species of the third group from the Salmonella and dysentery bacteria.

Among the Salmonellae the failure of *S. typhi* to produce gas from any carbohydrate separates it from the paratyphoid and food-poisoning organisms; and in the dysentery group the acidification of *mannitol* by the Flexner varieties, and of both *mannitol* and *lactose* by *Bact. dysenteriae* (*Sonne*) distinguishes them from the Shiga type and from each other. Numerous other fermentable substances are used for the finer differentiation of species and varieties; e.g. xylose, inositol,

saccharose, maltose, dulcitol, and also a range of organic salts such as tartrates, citrates, and fumarates.

The non-liquefaction of gelatin by all the genera with a few unimportant exceptions is a valuable point of

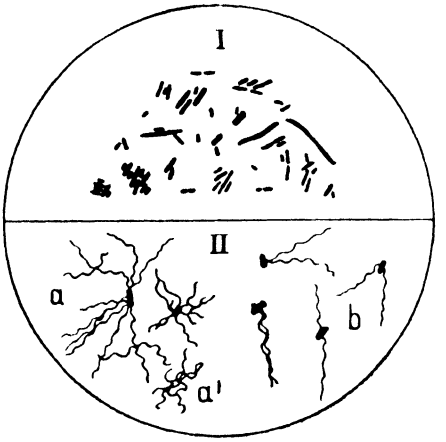

FIG. 11. Bacterium

I. A typical bacterium, e.g. *Salm. typhi* or *Bact. coli*.
II. Flagella of *Salm. typhi*. a. Orthodox view (peritrichate). b. Pijper's view (laterally amphitrichate—probably true). a'. Tangle of loose flagella.

distinction from *Proteus* and *Pseudomonas* (Table X, p. 101); and the failure of the Salmonellae to form indole and to coagulate milk distinguishes them from *Bact. coli*.

The Salmonella Group

(*Salmonella typhi*, *S. paratyphi A*, *B*, *and C*, *S. enteritidis* (*Gaertner*) and rarer species. Previously called Bact. typhosum, paratyphosum, &c.)

The average typical cell is a straight motile rod 2 to 3μ long and about 0·6μ broad. Much variation,

TABLE VIII

SPECIES	MORPHOLOGY: *Form*	*Motility*	*Spores*	*Capsules*	STAINING: *Gram*	*Acid-fast*	BIOCHEMICAL: *Oxygen*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Salmonella typhi . . .	Short rods: occasional filaments	+	0	0	0	0	AER	0	A then N or Alk.	Ordinary media Lactose O Glucose A Mannite A
Salmonella paratyphi A .	,,	+	0	0	0	0	AER	0	A	Ordinary media Lactose O Glucose AG Mannite AG
Salmonella paratyphi B & C *Salmonella enteritidis (Gaertner)* *Salmonella typhimurium* .	,,	+	0	0	0	0	AER	0	Sl. A, then Alk.	Ordinary media Lactose O. Glucose AG Mannite AG
Bacterium dysenteriae (Shiga)	,,	0	0	0	0	0	AER	0	Sl. A, then N	Ordinary media Lactose O Glucose A Mannite O
,, ,, (*Flexner*)	,,	0	0	0	0	0	AER	0	Sl. A, then N	Ordinary media Lactose O Glucose A Mannite A
,, ,, (*Sonne*) .	,,	0	0	0	0	0	AER	0	Late strong A, often C	Ordinary media Lactose, late A Glucose A Mannite A

A = acid; N = neutral; Alk. = alkaline; AER = aerobic; G = gas; + = positive; O = negative.

however, occurs, and long unsegmented filaments are to be found in most cultures. *On agar* in 24 hours at 37° C. all species produce a greyish-white, smooth, moist film of growth, or round, smooth colonies of good size (2 to 3 mm.). *S. typhi* and *paratyphi A* grow rather less profusely than the others.

Long-cultivated races tend to throw a proportion of rough variants (p. 44). A few permanently non-motile species, and occasional non-motile variants of motile species, are met with in the group.

In broth the normal, smooth type grows with uniform turbidity and at first little or no deposit.

Litmus milk. In the case of *S. paratyphi B* and *C*, *enteritidis*, and *typhimurium* (aertrycke) a slight acidity in the first 24 hours changes to neutral and then alkaline in two or three days; whereas *S. paratyphi A* continues acid for at least a week. *S. typhi* behaves at first like *S. paratyphi A*, but its reversion to neutral, or sometimes to an alkaline reaction, is generally earlier.

None of the group produces acetyl-carbinol (Voges-Proskauer reaction), but all except *paratyphi A* produce H_2S, which blackens lead acetate added to the medium.

Antigenic structure and variation in the Salmonella group. By means of exhaustive agglutination and absorption tests of all the known species, both in the natural state and after treatment with heat or chemicals, the following facts have been established:

(1) *Flagellar (H) and somatic (O) antigens.* With few exceptions the Salmonellae, being motile, have both H and O antigens (pp. 28, 100). In the few non-motile species and in non-motile variants of motile species only the O antigens are present. Serums made by injecting animals with such strains show only granular agglutination.

(2) *Diphasic alternation of flagellar antigens.* Most

of the species of this group have two alternative flagellar antigens, the one *specific*, the other *non-specific* (i.e. giving group-agglutination only). In each cell of a culture one of these antigens preponderates, to the

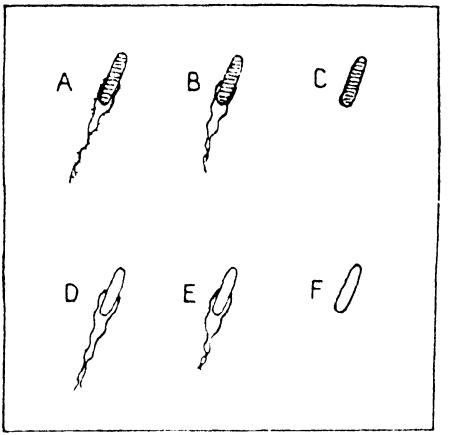

FIG. 12. Diphasic and rough-smooth variations of a Salmonella bacterium (diagrammatic)

A. Smooth motile specific. B. Smooth motile non-specific. C. Smooth non-motile (O type). D. Rough motile specific. E. Rough motile non-specific. F. Rough non-motile.

practical exclusion of the other. Thus, on plating out such a culture, some of the colonies are agglutinable only by a serum specific for that species, whereas other colonies are agglutinable also by the serums of related species. The position is made clear in Fig. 12 and Table IX. After a short period of cultivation a pure specific or non-specific sub-race again becomes mixed, by the production of cells in the alternative phase.

When injected into rabbits the specific phase produces chiefly specific agglutinins, the non-specific

phase a chiefly non-specific serum. Those organisms that show this type of alternating variation are called *diphasic*; they include *S. paratyphi B* and *C*, and *S. typhimurium*. There are many other diphasic species, each with its own specific antigen and a common non-specific one, but their comparative rarity makes it

TABLE IX

Antigenic structure of the more important members of the Salmonella group (simplified)

	Monophasic Species			Diphasic Species		
Type of antigen	*S. enteritidis*	*S. typhi*	*S. paratyphi A*	*S. paratyphi B*	*S. typhimurium*	*S. paratyphi C (suipestifer)*
Flagellar or 'H' specific	g	d	a	b	i	c
Flagellar or 'H' non-specific	—	—	—	1	1	1
Somatic or 'O'	IX(Vi)	IX	I	IV	IV	VI

Note. Each symbol represents an antigen. Minor antigenic components are omitted.

unnecessary to describe them in detail. There are also a number of complicating factors which we need not discuss here.

Monophasic species are those like *S. typhi*, *S. paratyphi A*, and *S. enteritidis*, whose single flagellar antigen is of the specific type (Table IX). Another kind of monophasic species exists, though it does not include any of the most important organisms. Here it is the specific phase that is lacking, the only flagellar antigen being of the non-specific type.

(3) *Smooth and rough phase-variation.* The fundamental change underlying the *rough* variation (p. 44) is the loss of a somatic component present in the

original smooth phase. The rough, however, still possesses a somatic antigen of its own. Different antibodies are, therefore, evoked by injection of the two variants into animals.

The rough phase, like the smooth, is usually motile, and is then subject to the diphasic variation of its flagellar antigen. But it is sometimes devoid of flagella; and since, as we have seen, it is also devoid of the somatic antigen which makes for smoothness, it may be regarded as having an irreducible minimum of antigens (Fig. 12). The rough somatic antigen is the least specific of all. Thus, cross-agglutinating relationships between the rough variants of different groups in the genus *Bacterium* have been established, and are taken as evidence of genetic relationship between species that are otherwise very unlike.

Important relationships. The main somatic antigens of *S. typhi* and *S. enteriditis* are identical, whereas their flagellar substances are distinct. *S. paratyphi B* and *S. typhimurium* are similarly related. In many cases where a main antigen is held in common by two species, minor components will allow a distinction to be established by absorption tests. The full antigenic scheme (of Kauffmann & White) classifies the 40 or 50 species into about 15 subgroups, according to their different O antigens. These are further subdivided according to their specific H components, and also to variable minor components of the nonspecific phase. The matter has reached the stage of complexity when even experts need a table of reference. Luckily, most species are rare, and the common ones are easy to identify with serums made with the specific H phases.

Enteric fever (typhoid and paratyphoid)

This is an acute fever with early invasion of the bloodstream, followed by an ulcerative inflammation of the

Peyer's patches and solitary follicles of the intestinal mucosa. Haemorrhage into the bowel and intestinal perforation often result. The cellular infiltration consists chiefly of large mononuclear cells with some polymorphonuclear leucocytes. The enlarged mesenteric lymph-nodes and the spleen show similar histological changes. Inflammatory rose-spots in the skin and focal necroses in the liver indicate a wide dissemination of the bacteria, which also invade and multiply in the gall-bladder. Occasional abscesses occur in the periosteum of bones and in other organs in the later stages of the illness. Typhoid fever (due to *S. typhi*) is generally more severe and has a greater mortality than the paratyphoid fevers.

Channels of infection. (1) *Drinking water* contaminated with sewage. (2) *Milk* contaminated by a human carrier. (3) *Moist foods* infected by a carrier or by flies that have recently fed on contaminated excreta. (4) *Direct contact* with enteric patients or carriers. (5) *Shellfish* from contaminated estuaries. A carrier is a person who has had an attack of the disease and continues to harbour the microbe in his gall-bladder or (rarely) kidneys, whence it is discharged intermittently in the faeces or urine respectively. The carrier-state may be of short duration, but often lasts for years or even for life. It can often be cured by excision of the gall-bladder.

In the British Isles enteric fever is due almost exclusively to *S. typhi* and *S. paratyphi B.* Of the other two paratyphoids 'A' is endemic in parts of Asia and Africa, and 'C' (suipestifer) is a somewhat rare cause of atypical enteric in warm climates. Two types of *S. suipestifer*, closely related to *S. paratyphi C*, are commonly found in acute intestinal infections of swine.

Toxins and antibodies. The poisonous action of these

organisms is due to *endotoxins*, consisting of, or including, specific carbohydrate-phosphatide antigens. Similarly connected with virulence is the minor component 'Vi' of *S. typhi* (Table IX), which overlies the main O antigen of virulent strains and protects them from agglutination by O agglutinin (at 37°, not at 52° C). Sufficient doses of either the living or the dead bacterium will kill small laboratory animals; but the disease caused in animals other than apes is a pure septicaemia, quite unlike enteric fever in man. Many instances of infection with pure cultures of *S. typhi*, accidentally swallowed in the laboratory, have given complete proof of its causal role.

The body reacts to the infection by producing specific antibodies against the various antigens of the organism. Antibody-production begins on the 4th or 5th day of the illness, and reaches its maximum in 2 to 3 weeks. Immunity against reinfection with the same bacterial species generally lasts for a good many years.

Diagnosis depends on cultivation of the bacterium from the blood, faeces, or urine, and on the 'Widal' reaction, which is a test for the presence of specific agglutinating antibodies in the patient's blood-serum. The all too common practice of relying solely on the Widal reaction is to be deplored.

For *blood-cultivation* about 5 to 10 c.cm. of blood are obtained by syringe-puncture of a vein of the fore-arm and a few cubic centimetres are introduced into 5 c.cm. of ox bile or one or two small flasks containing 50 to 100 c.cm. of broth. After suitable incubation a loopful of the primary culture is streaked on agar or some other suitable medium, and any colonies that develop are put through the various identification tests.

For *stool-cultivation* a specimen of faeces is diluted with broth and plated out on a special lactose-containing agar medium (e.g. McConkey or Endo), on which

Bact. coli produces coloured colonies, and the non-lactose-fermenting Salmonellae give uncoloured ones. There are many variations and refinements of this method, for which larger works must be consulted.

Cultures from *urine* are made by centrifugalization and plating the deposit on McConkey's or a similar medium.

Identification of bacteria. Colourless colonies on the special medium are put through the biochemical tests indicated on pages 80 and 81. Any whose reactions correspond with those of the known pathogenic bacteria are finally identified by agglutination with specific serums.

The Widal reaction. About 2 c.cm. of the blood drawn from the vein for blood-cultivation is put into a separate sterile tube, and after coagulation the serum is pipetted off. Dilutions of serum ranging usually from 1 in 10 to 1 in 100 are made with normal saline solution in small tubes, one set for each type of bacterial suspension to be tested, and a constant volume of the suspension is then added. After incubation in a water-bath at 50–55° C. (H. suspensions 2 hrs.; O suspensions 24 hrs.), the highest total dilution showing good clumping is recorded as the 'titre' of the serum. If the range of serum-dilutions is insufficient, a second set from 1 in 200 to 1 in 2,000 is made. The result may be taken as 'positive' if the titre is three or more times higher than either (*a*) the average titre of natural agglutinins for that microbe in the country concerned, or (*b*) the titre shown by the patient in an earlier test, before agglutinin-production became active. The latter criterion, involving repeated tests, is the sounder.

For the test in western Europe the following suspensions are necessary: (1) *S. typhi* (*a*) flagellar (H), best made by diluting a well-grown broth culture one in two with saline solution containing 0·4 per cent of

formalin, (*b*) non-flagellate (O) variety, similarly formolized (or motile bacteria treated with alcohol to destroy the flagella). The H suspension detects and measures the flagellar agglutinins, the O suspension the somatic, which are often developed earlier in the disease, and sometimes are the only agglutinins present. (2) For the paratyphoid infections, generally speaking, only 'H' suspensions are necessary, since flagellar agglutinins seem hardly ever to fail. The test should include (*a*) *specific* 'H' suspensions of each type (A, B, and C,) and (*b*) a *non-specific* 'H' suspension of a diphasic species such as *paratyphi B* or *typhimurium*, in case the group-agglutinin alone should be present.

The test is partly vitiated by *previous attacks* of enteric fever and by *preventive inoculation*, both of which produce both flagellar and somatic agglutinins. Since the flagellar 'H' agglutinins may persist in the blood at a considerable titre for many years, agglutination of the motile suspension in such cases cannot be interpreted as evidence of present infection. But it happens that the anti-somatic (O) agglutinins due to inoculation (and probably also to past infection) soon sink to titres less than 1 in 50, and, therefore, an 'O' titre much above this level is a good indication of active infection. Since, however, the somatic antigens of the different species have a common component, not shown in Table IX, it may be impossible to decide which of the enteric group is involved.

A further trouble with inoculated persons is the *anamnestic reaction* which occurs in typhus fever and certain other febrile states. It consists of a sharp rise of the residual 'H' titre of the patient's serum against *S. typhi* (and to a less degree against the paratyphoid bacteria) during the acute stage of the fever. The 'O' titre, however, is seldom much raised; so that a negative reaction with a typhoid 'O' suspension together

with a positive Weil-Felix test (p. 102) will identify the disease as typhus fever.

Carriers are detected by plating out the stools. Excretion of the bacteria is so intermittent that several repetitions are necessary. The agglutination test is useful for sorting groups of suspects. Since carriers invariably show some agglutinins, a completely negative reaction (H and O) at 1 in 10 removes the subject from the group requiring stool examination. A positive reaction does not, of course, prove the carrier-state.

Preventive and therapeutic inoculations. Prophylactic inoculation with killed bacterial suspensions (vaccines) has been shown to induce a considerable degree of immunity for at least a year. In the Boer war, when inoculation was not practised, more men died of enteric fever than of wounds, whereas in the Great War of 1914, when it was carried out thoroughly, the death-rate from enteric was negligible. At first only *S. typhi* was used, but later *S. paratyphi A* and *B* were added to the vaccine, since it became clear that the typhoid vaccine did not afford as much protection against paratyphoid as against typhoid fever. The fact that it apparently did afford some protection is attributable to the minor common O component already mentioned. The statistics compiled in the war, though indicating the great value of inoculation, are too imperfect to prove it scientifically. Convincing figures, however, were already on record; one of the best examples being a 1913 report on British troops stationed abroad:—Of a total of 18,986 men, 8,664 who had not been inoculated experienced 272 attacks of typhoid; whereas 10,332 inoculated men had only 52 attacks. The odds against this difference being due to chance are more than 10,000 to 1. Much corroborative evidence was obtained in other armies during the war, and has been confirmed in numerous later epidemics.

Recent advances in our knowledge of antigens have shown that it is essential to make vaccines with the smooth phase of the bacteria, since it is the smooth somatic antigens that produce immunity, while antibodies against the flagellar or 'H' antigens are of little or no importance.

Oral immunization. The administration of prophylactic vaccines by the mouth has been tried on a considerable scale. The statistical evidence so far collected is favourable, and is supported by the fact that persons thus treated develop the important anti-somatic agglutinin: but further evidence of its value is still needed (see p. 249).

Gastro-enteritis

This variety of 'food-poisoning' is caused by various species of the Salmonella group. *S. enteritidis* (*Gaertner*) and *S. typhimurium* are by far the most common; but a great number of species or varieties, often distinguishable only by the possession of special specific antigens, have been isolated from single cases or small epidemics.

The symptoms of gastro-enteritis are those of acute poisoning. With little or no incubation period an attack of vomiting and diarrhoea sets in, lasts for a day or longer and usually passes off without serious results. Fatalities, however, are by no means rare.

The microbes are usually swallowed in considerable numbers with the contaminated food in which they have been growing, which is usually mince or sausage-meat prepared in bulk and unhygienically stored before use.

The sources of infection are: (1) The animal providing the mince-meat. (2) Rats, mice, or human carriers infecting the meat. (3) Eggs of infected ducks.

Many animals and rodents suffer from natural Sal-

monella infections, and *the human disease is essentially of animal origin*. Rats are so susceptible to *S. enteritidis* and *S. typhimurium* that commercial preparations of living bacteria of these types ('rat-virus') are used for rat-extermination. But the claim of the vendors that these 'viruses' are free from risk to human beings and domestic animals has more commercial than scientific justification.

The *toxins*, which are heat-resistant, are not present in filtrates of young cultures, and are therefore classed as endotoxins. Owing to their presence, contaminated meat can cause severe symptoms even after it has been sterilized by cooking. Here the symptoms are immediate, as in botulism (p. 173).

Diagnosis. This is generally done by cultivation of the microbe from the stools or vomit. Blood-cultivation is much less reliable than in the enteric fevers, though the organism is often present in the blood in fatal cases before death.

Platings of a suspension of faecal matter on a lactose-containing medium are examined for non-lactose fermenting colonies, and these, if present, are sown in various carbohydrate media and a culture in broth or on agar is made to provide a suspension for agglutination. The test is done with specific 'H' serums of *S. enteritidis* and *S. typhimurium*, and with a composite group-phase Salmonella serum. If these give negative results, serums of the less common types may be tried, but the accurate identification of rarer types can only be done in special laboratories.

The agglutination-test of the blood-serum of the patient is capable of giving the diagnosis clearly; but the test is only applicable in protracted cases, or in convalescence for the purpose of retrospective diagnosis, since an ordinary acute attack is over long before the agglutinins develop. The serum is tested against

suspensions of the specific phase of *S. enteritidis* and *typhimurium* and a non-specific phase Salmonella suspension. If the two former are negative, further tests are done against the specific phases of the rarer species.

THE DYSENTERY GROUP

The chief members of this group are: *Bacterium dysenteriae* (*Shiga*), *Bact. dys.* (*Flexner*), and *Bact. dys.* (*Sonne*). Two salient characters distinguish the dysentery bacteria from the majority of the Salmonella group and *Bact. coli*, viz. *lack of motility* and *failure to produce gas* in the fermentation of carbohydrates. There is nothing characteristic in their morphology or cultural requirements. They are found chiefly in the thin, purulent faeces in cases of acute and chronic dysentery, and only very rarely in the healthy human intestine, or in animals. In the outer world they occur from time to time in contaminated water, milk, and sometimes in soft foods.

The three main species are distinguishable from one another not only by their *biochemical actions* (p. 80), to which we may add the production of *indole* by the *Flexner* species alone, but also by their *antigenic specificity*.

Antigenic properties. The non-mannite fermenting *Shiga* is an antigenically distinct and uniform species with a single, specific, smooth antigen. This, of course, is somatic, since we are dealing with a non-motile group of organisms.

The *Flexner* species or sub-group contains a number of antigenic varieties, each having a specific and also a variable quantity of a group-antigen. During cultivation the latter tends to supplant the former. Various names have been given to specific strains; V, W, X, Z, 103, 119, &c. The type called Y has lately been shown to be an entirely non-specific form, derivable probably

from any specific type. There is practically no cross-reaction with the *Shiga* or *Sonne* species. The last-named (*Sonne*) is antigenically uniform, and quite distinct from the others, apart from a minimal cross-reaction with the X type of *Flexner*.

Rough non-toxic variants occur in old cultures of all species, and differ antigenically from the smooth phase owing to the loss of the specific smooth component. There is, as usual, more cross-reaction amongst the rough variants than amongst the smooth (cf. p. 84).

In this connexion the *Sonne* bacterium has an interesting peculiarity. Direct platings from dysenteric faeces in this infection usually show only smooth colonies, but it is not uncommon to see also flatter, granular, spreading colonies, apparently of the rough type. When a smooth colony is cultivated in broth and then plated out, a large proportion of these rough colonies will almost always develop, and unless special precautions are taken, the whole culture soon turns rough.

Pathogenic action and toxins. The normal smooth phase of all three species is *toxic* in different degrees to animals (rabbit, guinea-pig, horse, &c.) when injected intraperitoneally or intravenously in considerable doses; but no actual infection takes place. Of the three, *Shiga* has by far the strongest action, the other two being about equal. These latter are often called the 'atoxic' or even the 'pseudo-dysentery' bacteria. The toxins of the mannite fermenting species, *Flexner* and *Sonne*, are endotoxins, but that of *Shiga* is of an intermediate type (p. 49), having some of the characteristics of an exotoxin.

The *lesion* of bacillary dysentery in man is an inflammatory ulceration of the mucous membrane of the large intestine, with swelling of the regional lymph-nodes and general intoxication. The outstanding

symptom is a violent bloody diarrhoea. The bacteria do not generally invade the body widely, and are rarely to be found in the blood. Though the malady is usually short and sharp, chronic ulceration is not uncommon. This either manifests itself as 'chronic ulcerative colitis', or, if of minimal extent and consistent with moderate health, gives rise to the *carrier-state*, which is often responsible for the spread of infection.

Shiga dysentery in man is generallly more severe than infection with the 'atoxic' species, though the latter is often serious and occasionally fatal.

Asylum dysentery and some epidemics of infantile summer diarrhoea are due to one or other of the mannite fermenting 'atoxic' species.

Routes of infection. Dysenteric infection enters by the mouth. The common channels of spread are: (1) Direct infection with excreta. (2) Faecal contamination of drinking-water. (3) Contamination of milk or food by flies or carriers. Experimental infection of human volunteers with pure cultures of *Bact. dys.* (*Shiga*) has fully proved its aetiological role; and accidental infections in the laboratory have done the same for the *Flexner* type.

Antibodies; immunization and serum-therapy. In the defensive reaction of the body to the infection, specific agglutinins and, in the case of *Shiga*, antitoxins are elaborated and can be detected in the blood; and the production of similar antibodies can be induced in horses by a careful course of injections of killed, followed by living, cultures. There is fairly good evidence of the therapeutic value of antidysenteric serum in the toxic (*Shiga*) type of infection. A polyvalent serum containing antibodies against *Shiga* and the main *Flexner* types is commonly used, but its effect on *Flexner* infections is problematical; while *Sonne* generally causes too mild a disease to need specific treatment.

Active preventive immunization has been practised both with subcutaneous injections and by oral administration of killed vaccines, and considerable success has been claimed, but no convincing statistics are available. *Vaccine-therapy* and treatment with specific bacteriophages (p. 224) are in a similar position.

Diagnosis. Microscopic examination of bloody mucus from the stools shows numerous polymorphonuclear leucocytes, red cells, and swarms of non-motile Gram-negative bacteria. The absence of *Entamoeba histolytica* excludes the symptomatically similar amoebic type of dysentery (p. 197).

Plate-cultivation of perfectly fresh stools in the early stages generally yields numerous non-lactose fermenting colonies with the biochemical and agglutinative characters of the group.

Finally, the *agglutination-reaction* of the patient's serum may give evidence of the production of specific antibodies. At least four suspensions must be used for a complete test: (1) *Shiga*. (2) *Flexner* V, W, and Y mixed. (3) *Flexner* X and Z mixed. (4) *Sonne*. With a carefully controlled technique the method is capable of giving a diagnosis in a good proportion of cases, especially in *Shiga* infections; but the titres are usually low, and in many cases no rise of agglutinins can be detected, so that the method is less generally practised than the Widal test for enteric fever. Normal human serum sometimes agglutinates *Flexner* suspensions to 1 in 50 or 1 in 100; and the rough type of *Sonne* to 1 in 200 or more.

Rarer species of the dysentery group. Bact. dys. (*Schmitz*), a non-mannite fermenting, but indole-producing species, is accepted on good evidence as causing occasional cases and epidemics of dysentery. The same is true of *Bact. Newcastle* (or *Flexner 88*), an irregular mannite fermenter with a variable power of producing

gas in glucose. Both the foregoing species have specifically distinct antigens. Two other biochemically and antigenically distinct species, *Bact. alkalescens* and *Bact. dispar*, are suspected of causing dysenteric symptoms.

CHAPTER VII

BACTERIUM (cont.). PROTEUS; PSEUDOMONAS; VIBRIO

Bacterium coli (Table IX)

THIS name covers a group of lactose-fermenting rods with the general characters of the genus *Bacterium* (p. 78). Most of them normally inhabit the intestines of animals and man and are also found in water and soil contaminated with excreta. The group interests us in three ways:

(1) As the regular intestinal commensals from which we have to separate and distinguish the pathogenic organisms in cases of intestinal disease.

(2) As containing a sub-group of varieties which are capable of causing disease, especially of the urinary tract.

(3) As affording evidence of the faecal contamination of water-supplies (see p. 261).

The varieties included under the general term *Bact. coli* are distinguished from each other by their fermentation reactions and differences of motility; also by their behaviour in the *Voges-Proskauer reaction* (addition of strong NaOH to a 3 days' glucose peptone-water culture gives an eosin-red colour due to acetyl-methyl-carbinol); by the pH they produce in a glucose medium (methyl-red test); and finally by the ratio of CO_2 to H_2 produced from carbohydrates (gas-ratio). Tables of these reactions will be found in larger text-books.

The *fermentation of lactose*, quickly and with gas-production, seems to be a peculiarity of the non-pathogenic or saprophytic varieties, whereas those races that have either been proved or suspected to be

pathogenic are generally atypical in the slowness of their lactose-fermentation or in their failure to produce gas. In other words, adaptation to the commensal type of nutrition develops certain ferments which are suppressed in variants that have acquired the power of tissue-invasion. It is tempting to go one step farther and speculate that the highly pathogenic Salmonella and dysentery bacteria may have originated from some ancestral coliform stem by a series of adaptations involving, among other changes, the complete suppression of the lactose-splitting ferment. Evidence that the faculty is not entirely lost in highly pathogenic species, such as *Bact. typhosum*, has been given by the successful 'training' of races of that organism to ferment lactose by prolonged cultivation in lactose-containing media. A particularly interesting type of late lactose-fermentation is a central feature of the peculiar colony-variation of a race called *Bact. coli mutabile*. The colonies of this organism on a lactose-containing agar with an indicator dye which turns red on acidification are at first all colourless, but after two or more days incubation some or all of the colonies show the development of numerous tiny knobs or 'daughter' colonies, which grow larger and then turn pink or red, indicating lactose fermentation. A fresh plating from such a colony after 24 hours incubation shows a mixture of primary red colonies and colourless ones, which later produce red knobs. The former are quick lactose-fermenting variants of the slow-fermenting mother race.

A similar type of variation is seen in *Bact. dys.* (*Flexner*) and other organisms grown on carbohydrate media.

The late lactose fermenters are mostly found in diarrhoeic or dysenteric disorders of the intestines, especially in the tropics, and are thought to be at least a contributory cause of the condition.

Finally, the *haemolytic* type is definitely pathogenic, being found exclusively in infections of the urinary tract cystitis, cysto-pyelitis, and the like. It can be readily cultivated from the purulent urinary sediment and identified by its fermentation reactions.

Antigens. The somatic constituents of different races are very diverse, the haemolytic group alone having at least ten different O antigens. Little seems to be known about the flagellar antigens.

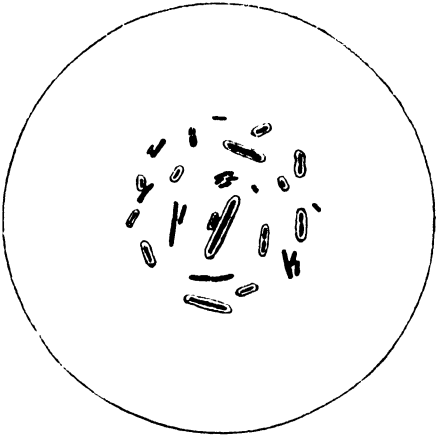

FIG. 13. *Bacterium friedländeri* (capsulated and uncapsulated cells)

Bacterium friedländeri

This is a short, stoutish *capsulated* 'coliform' organism strongly resembling *Bact. coli* in its cultural and biochemical reactions, and found in infections of the respiratory tract and in suppurative lesions elsewhere. It is also present in a small proportion of normal intestines (p. 101 and Fig. 13).

It is undoubtedly pathogenic, causing ozaena, rhinoscleroma, abscesses of the brain and kidney, and cystitis, and being frequently associated as a secondary infection with *Streptococcus pneumoniae* or *pyogenes* in bronchitis and broncho-pneumonia. (Hence its old name Bac. pneumoniae.) It occasionally gives rise to fatal endocarditis and septicaemia. It is highly virulent to mice and less so to other animals.

The capsule, which contains a specific polysaccharide (p. 48), is formed both in the tissues and in cultures. Growth on ordinary media is profuse and *mucoid* (i.e. semi-fluid and sticky) owing to the abundant moist capsular material.

Proteus (Table X)

This genus consists of highly motile Gram-negative, non-sporing rods of variable length, not fermenting lactose, but producing acid and gas in some other carbohydrates, and showing strong proteolytic activity.

Commonly found as saprophytes in soil, water, and in the intestines of animals, the group interests us as potential causes of suppuration or intestinal irritation, and as providing by a curious accident of antigenic structure a diagnostic reaction for typhus fever, the true cause of which (*Rickettsia*, p. 210) has no known connexion with *Proteus*.

The name *Proteus* is derived from a supposedly protean morphological variability, chiefly in the length of the rods. The chief cultural peculiarity of the group is a tendency to spread rapidly ('swarm') over the surface of solid media. The annoyance of finding plate cultures of water or faeces spoiled in this way is familiar to bacteriologists.

H and O variation of colonies. In addition to the spreading centres of *motile* or H growth, which tend to produce a confluent film, many cultures show circum-

TABLE X

SPECIES	MORPHOLOGY				STAINING		BIOCHEMICAL			
	Form	*Motility*	*Spores*	*Capsules*	*Gram*	*Acid-fast*	*Oxygen*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Bacterium coli	Short rods and occasional filaments	+ (var.)	0	0	0	0	AER	0	AC	Ordinary media Lactose AG Glucose AG Mannite AG
Bacterium friedländeri	Do.	0	0	+	0	0	AER	0	AC (var.)	Same as above
Proteus vulgaris	Rods of var. length	+	0	0	0	0	AER	+	CP	Ordinary media Lactose O Glucose A (G) Mannite AG
Pseudomonas pyocyanea	Short rods and occasional filaments	+	0	0	0	0	AER	+	CP	Ordinary media Lactose O Glucose (A) Mannite O
Vibrio cholerae	Short curved or S-shaped rods	+	0	0	0	0	AER	+	O or sl. A (C) P	Ordinary media (likes alkalinity) Mannose A Saccharose A Arabinose O

A = Acid. Alk. = Alkaline. C = Clot. G. = gas. P. = peptonization. Var. = varies. AER = Aerobic.
( ) = Late or inconstant. 0. = no action.

scribed round colonies without any tendency to spread; and microscopic examination shows these to be *non-motile* or O variants.

Weil-Felix test for typhus fever. The blood of typhus fever patients contains agglutinating antibodies which act on the *somatic antigen* of certain *Proteus* varieties, particularly one known as *Proteus X 19*. The non-motile (O) form of the organism is used, since it is free from the irrelevant flagellar antigen of the H-form (p. 23).

The technique of the test is the same as that of the Widal reaction (p. 87). In the Malay States, India, &c., a race known as *Proteus XK* has to be used, since the agglutinins formed in response to the local *Rickettsia* act on *XK* antigens only. This double identity of antigenic fractions in two varieties of *Rickettsia* and two of *Proteus* is an interesting mystery.

The Weil-Felix reaction is positive in the vast majority of typhus cases after the first few days of illness, and negative in normal persons. A difficulty in the interpretation of the combined Weil-Felix and Widal tests, when a diagnosis between typhus and enteric has to be made in typhoid-inoculated persons, is described on p. 88 under the name of the *anamnestic reaction*.

Proteus infection in man is seldom primary; that is, the organism is generally found in association with more pathogenic bacteria in suppurative conditions such as cystitis, peritonitis, otitis media, and in various forms of diarrhoea. But it has also been occasionally reported as the primary agent in pyelonephritis and septicaemia.

Most varieties of *Proteus* are pathogenic in fairly large doses to small animals, causing a fatal general infection with haemorrhagic diarrhoea and intestinal abscesses. The action appears to be mainly or wholly endotoxic.

'Morgan's No. 1 bacillus' is a small Gram-negative motile rod often found in, and probably causing, infantile diarrhoea. Whereas it has hitherto been allotted to the genus *Bacterium*, its recently discovered power of 'swarming' on moist agar and its possession of an H antigen in common with *Proteus* suggest its allocation to this genus.

Pseudomonas

(Syn. Bacillus pyocyaneus, fluorescens, &c.)

This is a genus of highly motile small Gram-negative rods, rather like *Proteus*, but distinguished by the production of greenish or bluish pigments.

Only one species, *Pseudomonas pyocyanea*, the microbe of blue pus, is pathogenic; the others, which we may class together as *Ps. fluorescens*, are proteolytic saprophytes of water and soil.

Pseudomonas pyocyanea (p. 101) is found in suppurative conditions of man, usually associated with other pyogenic organisms. It is especially common in the old discharging sinuses of bone disease (tuberculosis: mastoiditis). The pus is pale bluish-green, and has a curious aroma. The organism also occurs in diarrhoea and dysenteric disorders, and in cystitis. It occasionally invades the blood, causing a typhoidal septicaemia; or the brain, causing meningitis. In the outer world it is not uncommon in soil and water contaminated by excreta. The pathogenicity of the organism to small animals is generally similar to that of *Proteus* (p. 102).

The *pigment* consists of varying quantities of two components: the non-fluorescent greenish-blue, *pyocyanin*, soluble both in water and chloroform; and the yellowish *fluorescin*, soluble only in water. *Ps. fluorescens* produces only the latter.

Vibrio

(*Asiatic Cholera*)

This genus of Gram-negative, motile, non-sporing, unencapsulated, curved rods contains one species of great importance, *Vibrio cholerae*, the cause of cholera. Very numerous saprophytic species are found in water, soil sewage, and in human intestines in the East. A few of these are supposed to be capable of causing severe diarrhoea (paracholera), but the question needs reinvestigation. Another species, *Vib. metchnikovi*, causes a natural infection of birds.

Vibrio cholerae

(Koch, 1886)

This organism is chiefly found in the excreta of persons suffering from cholera. It may also be present in contaminated water, soil, or sewage.

The main characters of the organism are given on page 101 and Fig. 14. Its motility is due to a single terminal flagellum. The vibrio shows a considerable variation of form. In the fluid stools of cholera and in broth cultures of recently isolated races the characteristic short, curved 'comma' is abundant, and double-curved or sigmoid cells are often seen. In old cultures spirally twisted longer filaments are not uncommon, and branching Y-forms (p. 43) may sometimes be found. Stock laboratory races tend to lose their curvature and are not always easy to distinguish from the straight rods of *Bacterium*, *Proteus*, &c. An important peculiarity of the organism is its preference for *strongly alkaline media*. This is useful for isolating the organism from stools containing other bacteria, since incubation in peptone water at a pH of about 8·5 selectively encourages the vibrio, which develops

both diffusely in the fluid and as a film or pellicle on the surface.

The colonies on solid media cannot be distinguished from those of the genus *Bacterium*, though they tend to be more transparent. Old colonies and stabs in

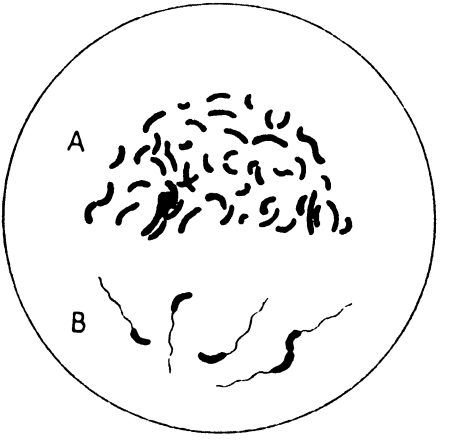

FIG. 14. *Vibrio Cholerae*
A. Ordinary examination.
B. Stained for flagella.

agar take on a brown colour; and old growths on potato show yellowish, brown, or pink pigmentation.

Biochemical actions. The production of proteolytic enzymes is shown not only by the liquefaction of gelatin but in the digestion of coagulated serum and in the peptonization of milk. *Vib. cholerae* produces acid, without gas, in a characteristic range of carbohydrates (p. 101), which greatly helps identification, though a few other vibrios show the same range. It produces *indole* and nitrites; and a compound of the

two, *nitroso-indole*, can be demonstrated in peptone-water cultures by adding a few drops of pure H_2SO_4 or HCl. This is the well-known *Cholera-red reaction*, which, however, is not specific for *Vib. cholerae*, but is shared with a number of paracholera and other vibrios.

Haemolysis. The true *Vib. cholerae* does not haemolyse red-cells, but the non-pathogenic variant known as the *Vibrio El Tor* has this property, as also have many other harmless vibrios.

Antigens. The flagellar antigen of *Vib. cholerae* is not distinctive, being common to the genus *Vibrio*, or at least to a large part of it. But by means of an anti-somatic (O) agglutinating serum the cholera vibrio is sharply distinguishable from the rest, excepting *El Tor*. The O antigens contain specific polysaccharide haptens.

Pathogenicity, toxins, &c. The cholera vibrio causes fatal septicaemia in small animals when injected into the blood or peritoneum, and it can give rise to a violent cholera-like enteritis. Typical cholera in man has several times followed accidental laboratory infection, and also intentional self-infection (e.g. Pettenkofer and Emmerich's attempt to disprove the causative character of the vibrio).

The invasion and intoxication are attributable to the endotoxin, which is probably the O antigen.

The disease in man is a violent irritative inflammation of the intestinal tract, especially the small intestine, resulting in the almost incessant discharge of liquid ('rice-water') stools containing blood and flakes of mucus. Vomiting, prostration from lowered blood-pressure (dehydration), and other symptoms of intoxication as often as not end in death.

The microbe invades the mucous membrane as far as the submucosa, causing acute inflammation and

patchy necrosis. The contents of the small intestine sometimes yield a pure culture of the vibrio. The bile-passages are usually invaded, and degenerative changes are seen in liver, kidneys, lymph-nodes, and other organs, from which the organism can be cultivated *post mortem*. Pneumonia is not uncommon. The blood is usually sterile, though vibrios doubtless pass through it from time to time.

Routes of entry and spread. Entry is by the mouth. Although the vibrio cannot survive exposure to the full acidity of the gastric juice, it may slip through into the small intestine when swallowed with large quantities of water, and it will find there a favourable alkaline medium for growth. Direct contact with the illness and the consumption of fouled water or food are the commonest modes of spread. In India the movement of bands of insanitary pilgrims are often accompanied by a wave of cholera, the intensity of which, and its spreading powers, are greatly affected by climate, rainfall, temperature, the humidity of the soil, and the prevalence of flies.

The majority of those who recover from an attack continue to excrete the vibrio for some days or weeks, and a small proportion become chronic carriers, the organism continuing to propagate in the gall-bladder (cf. 'Typhoid', p. 85). Mere contact, without subsequent illness, may give rise to a temporary carrier-state.

Outside the human body the life of the organism is usually short, but it appears sometimes able to survive for several weeks in water.

Specific defence. If the patient survives the first few days, specific antibodies appear in the blood, demonstrable by agglutination and bactericidal tests (e.g. *Pfeiffer's reaction*, p. 240).

Prophylactic immunization of guinea-pigs by injec-

tions of dead or living vibrios protects them from the relatively small dose of *living* organisms required to kill a normal animal, but they can still be killed by a single lethal dose of *endotoxin* (i.e. a considerable mass of dead vibrios). This indicates that their immunity is antibacterial (anti-invasive) rather than antitoxic. *Protective inoculation* of man with heat-killed vaccines (suspensions of vibrios), preserved with phenol, is widely practised in the East, and statistics suggest that a valuable degree of protection results. Attempts have been made to produce *anti-endotoxic serum* in horses, but though these have a strong protective action on animals, their curative power is not very striking either in animals or man.

Diagnosis. The presence of numerous vibrios can generally be detected by examination of stained flakes of mucus from a 'rice-water' stool. If the mucus has not been much rubbed up, the vibrios may be seen to be arranged in it along parallel lines 'like fish in a stream'.

For the certain identification of the organism it must be isolated in pure culture by plating out on a suitable medium, such as Dieudonné's, which consists of agar containing a boiled mixture of alkali and blood. If the vibrios are not profuse, previous incubation of the faeces in alkaline peptone water is valuable.

The organism is identified by its cultural and biochemical characteristics, and finally by agglutination with a specific O serum. Pathogenicity to guinea-pigs by intraperitoneal injection excludes saprophytes, but not paracholera vibrios; and Pfeiffer's bacteriolytic test (p. 240) should be done when questions of public health rest on the diagnosis of an isolated case.

The serum-agglutination-test, owing to the shortness of the disease, first becomes positive in convalescence, and is therefore of very limited value in diagnosis.

TABLE XI

SPECIES	MORPHOLOGY				STAINING		BIOCHEMICAL			
	Form	*Motility*	*Spores*	*Capsules*	*Gram*	*Acid-fast*	*Organs*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Pasteurella pestis	Small ovoid rods; pleomorphic	0	0	0	0	0	AER	0	0	Ordinary media Slowish growth Acid in several carbohydrates
Haemophilus influenzae	Very small coccoid rod; some filaments	0	0	0	0	0	AER	0	—	*Needs X & V factors* Acid in several carbohydrates
Haemophilus pertussis	Very small ovoid rod, or 'coccobacillus'	0	0	0	0	0	AER	0	—	*Needs blood or serum only at first* Sugars not fermented Slow growth
Brucella melitensis	Small coccoid rods	0	0	0	0	0	AER	0	Sl. Alk.	Ordinary media Rather slow growth No fermentation
Brucella abortus	,,	0	0	0	0	0	AER	0	Sl. Alk.	Ordinary media, but *needs* CO_2 *at first* Rather slow growth No fermentation

CHAPTER VIII

PASTEURELLA. BRUCELLA. HAEMOPHILUS

PASTEURELLA

(*Plague*)

THIS genus consists of three species of small Gram-negative, non-sporing, unencapsulated, delicately growing parasitic rods, which give rise to serious generalized glandular and septicaemic infections in animals and man.

(1) The first, *Pasteurella pestis* (the plague bacillus), is the only medically important member of the group.

(2) The second, *Pasteurella pseudotuberculosis* (*rodentium*), which is very much like *Past. pestis*, but motile at low temperatures (see Table XII), causes a disease in rodents rather like tuberculosis, and may cause error in the diagnosis of plague in rats.

TABLE XII

Differential characters of the Pasteurella group

Species	*Growth in bile salt-media*	*Indole*	*Motility*	*Litmus milk*	*Acidification of Saccharose*	*Virulence for white rat*
Past. pestis.	+	—	—	Neutral	—	+
Past. pseudotuberculosis (rodentium)	+	—	+ (*below* 30° C.)	Alkaline	±	0
Past. aviseptica, &c.	—	+	—	Neutral	+	+

(3) The third species, which we may term *Past. septica*, consists of a number of varieties, each adapted to a different animal, not including man (cf. *Myc. tuberculosis*, p. 147), viz. *Past. aviseptica*, *boviseptica*, *muriseptica*, *lepiseptica*, and so on. These are also

termed the *haemorrhagic septicaemia group*, from the type of disease they produce.

The chief differential characters of these three species are given in Table XII, p. 110.

Pasteurella pestis

This species of small ovoid rods is found exclusively in the lesions of bubonic and pneumonic plague of human beings and rodents, and in the intestines of rat-fleas.

Its *morphological* and *cultural characters* (Tables XI and XII; Fig. 15) are not particularly distinctive, but the following points assist in its identification. Delicate, slowish growth; bipolar staining (p. 17) and chain-formation; pleomorphism (Fig. 15) in old cultures; and the production of a hanging, stalactite-growth under a layer of butter or oil floated on broth. None of these features are either absolutely constant, or exclusively confined to *Past. pestis*.

The *antigens* of the organism are (1) a superficial (capsular) specific antigen, on which virulence largely depends, and (2) deeper group-component. Thus, an anti-plague agglutinating serum agglutinates *Past. pestis* to full titre, and the remaining species only in the higher serum-concentrations. Similarly, *Past. septica* and *Past. pseudotuberculosis* have each their own specific antigens in addition to the common component.

Pathogenicity. Plague is primarily a disease of rodents. In various quarters of the globe spontaneous epidemics break out in rats, tarbagans, gerbilles, spermophiles, and ground squirrels, which act as reservoirs of infection for man. The disease can also be readily transmitted to these creatures by the injection of small quantities of the microbe in pure culture. Guinea-pigs, rabbits, marmots, mice, and

monkeys are also susceptible, but most of the larger animals show considerable resistance, and birds are completely refractory.

Toxins are not demonstrable in culture-filtrates, but the dead bodies of the organisms have a strongly

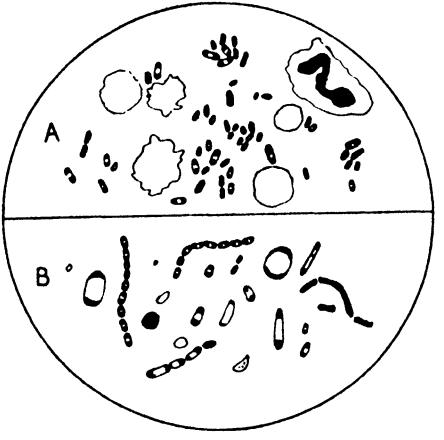

FIG. 15. *Pasteurella pestis*
A. Bipolar-stained rods in bubo-juice containing blood-corpuscles and other cells.
B. Chains and swollen involution-forms.

endotoxic action on animals, causing haemorrhagic necrosis at the site of injection and in various internal organs.

Plague in man. From the earliest historical times this terrible disease has swept intermittently through human populations, causing incalculable misery and destruction. In England a series of severe epidemics culminated in the Black Death of the fourteenth century, but the disease cropped up again repeatedly until two hundred years ago, since when there has

been only one small outbreak, at Glasgow in 1900. In the present era it ranks as an Oriental disease. In India alone between 1898 and 1918 the loss of life from plague was estimated at half a million a year; a number far exceeding the world's loss from wars and revolutions.

Transmission. It has been proved by the most exact experiments that the infection is transferred from rat to man by fleas. Just as rats are said to leave a sinking ship, so a rat-flea will leave a dying rat, and if no other suitable rodent is available, it will attach itself to a human being. Since its last feed consisted of rat-blood swarming with *Past. pestis*, its next bite is likely to be fatal. The experimental proof of the role of the flea is of the following kind. If guinea-pigs (or rats) artificially infected with plague are mixed with healthy guinea-pigs (or rats) under conditions which rigidly exclude fleas, the normal animals remain uninfected; but on the introduction of rat-fleas the disease immediately spreads. Again, a wide zone of sticky material painted on the floor of a hut containing infected fleas will completely protect from infection a monkey placed in its cage at the centre of the zone. It has been shown that the microbe multiplies in the flea's stomach and that the latter may still be infectious a fortnight after a contaminated feed.

Apart from fleas, the infection can be transmitted by human or rodent excreta; and in the pneumonic type it is spread direct from man to man by droplets (p. 6).

Plague occurs both in sporadic and in epidemic form. A human epidemic is preceded by an outbreak amongst rodents in one of the permanent endemic centres of rodent-infection in Asia, California, or South Africa. There is evidence that the infection of rodents has latterly tended to increase in various

parts of the world. In England it has never completely disappeared.

Two clinical types of the disease are recognized: *Bubonic plague* usually starts as a small skin-vesicle surrounded by a red area, very often on the leg. The regional lymph-nodes swell up into a large inflammatory mass, the bubo, which on section shows inflammation, haemorrhage, and necrosis. Other groups of lymph-nodes, axillary, inguinal, &c., undergo secondary enlargement. The blood-stream is often invaded, and a great enlargement of the spleen and degeneration of the liver and kidneys give evidence of widespread infection. Microscopic examination of the infected parts shows enormous numbers of microbes, and in about 30 per cent. of cases they can be cultivated from the urine. The fatality from this form of the disease varies from 60 to 90 per cent. of persons attacked.

In *pneumonic plague* the lesion is an acute bronchopneumonia with frothy or bloody sputum full of *Past. pestis*. Recovery is exceptional.

Immunity, prevention, and therapy. Although a certain degree of immunity can be induced in rodents with killed suspensions of the organism, and although their blood-serum has some bactericidal, opsonic, and protective action, attempts to produce efficacious serums in large animals for human therapy or prophylaxis have not been very successful.

A greater measure of success has met the attempts to produce *active, prophylactic immunity* by inoculation with killed vaccines. Haffkine's vaccine, which is the one most generally used, consists of 6 weeks' old broth cultures heated to 65° C. for an hour and preserved with 0·5 per cent. phenol. It contains both bacterial bodies and endotoxins liberated by autolysis. By inoculation with this vaccine white mice can be completely protected against the effects of otherwise lethal

doses of the living microbe. Although the value of prophylactic vaccination in man is disputed, the best available statistics point to a favourable action. The Indian figures up to 1916 are shown in Table XIII. The duration of the increased resistance following vaccination probably does not exceed a few months.

TABLE XIII

Protective inoculation against plague in India up to 1916

Population at risk	*No. of plague cases*	*Plague cases per 1,000*	*No. of deaths from plague*	*Plague deaths per 1,000*	*Per cent. survivors from attacks*
Uninoculated 3,230,000	59,500	18	50,700	15	15
Inoculated 1,940,000	4,100	2	1,600	0·8	61

It is likely that the efficacy both of vaccines and anti-serums may be improved by greater efforts to preserve the labile capsular antigen in the suspensions used.

General prophylactic measures. Any attempts to exterminate rats under present circumstances would be doomed to failure, but the spread of infection can be greatly diminished by attention to personal and domestic hygiene, house construction, and refuse disposal; in fact by all measures which tend to reduce the intimacy of contact between rodent and man.

In pneumonic plague the same prophylactic measures are applicable as in other droplet-spread diseases: disinfection of sputum, isolation of contacts, and the prevention of droplet infection of doctors and attendants by the wearing of gauze and wool masks.

Diagnosis. In the bubonic type the microbe is detected microscopically in juice obtained from the bubo or primary vesicle by needle-puncture, and is identified by cultivation followed by the agglutination-test with

specific serum. Another quick method is the direct inoculation of a healthy rat or guinea-pig with fluid from the bubo.

The pneumonic type is diagnosed by making Gram-stained preparations of the sputum, and by injecting a small quantity into a guinea-pig. Lung-puncture may be necessary if there is no sputum. Blood-culture is often negative in the earlier stages, but generally succeeds when the illness is well developed. Diagnosis after death, which may be necessary on public health grounds, can be done without an autopsy by aseptic puncture of the spleen and cultivation or injection into animals of the material obtained.

The identification of the organism in infected rodents, living or dead, follows the same principles, but in the final serological identification it must not be forgotten that agglutinating serum for *Past. pestis* often has a considerable action also on *Past. pseudo-tuberculosis*. Absorption and virulence tests may therefore be necessary.

Brucella

(*Mediterranean and 'abortus' fevers*)

This group or genus consists of small Gram-negative, non-sporing, unencapsulated, coccoid rods, which grow rather weakly on ordinary media and do not ferment any carbohydrates.

By far the most important species are *Brucella melitensis*, the cause of undulant Mediterranean or Malta fever, and the closely related *Brucella abortus* which is found in contagious abortion of cattle and swine, and in the comparatively rare undulant 'abortus' fever of man. On rather uncertain grounds, we include in the genus *Br. bronchiseptica*, a motile, alkali-producing rod, much like the others in form

and growth, which is found in catarrh and broncho-pneumonia of dogs, chiefly following distemper; and *Br. tularensis*, the cause of 'tularaemia,' a plague-like disease first observed in ground-squirrels in Tulare, California, which is transmitted to man by various wild rodents in America and E. Europe.

Brucella melitensis and Brucella abortus

The general characters of these two organisms are mainly of a negative type (Table XI p. 109).

Although the average shape of the cell is that of a short ovoid rod about 1μ long and 0·5μ broad, coccoid forms are so common that *Br. melitensis* used to be classed as a micrococcus. A peculiarity of the majority of races of *Br. abortus* from cows and man (not those from swine) is that on primary isolation they will only grow if 5 to 10 per cent. of CO_2 is added to the air in the culture tube. Later on they usually adapt themselves to growth without CO_2.

On agar rather small, greyish white, round, smooth, semi-transparent colonies develop in about 48 hours. Old growths tend to develop a brownish colour.

In broth there is feeble turbidity in 24 hours, but in a week or 10 days a dense culture develops, with a tenacious, ropy deposit and a strongly alkaline reaction.

On potato old cultures turn a deep chocolate-brown (cf. Pfeifferella, p. 140). In fermentation-tests, which are all negative, slight alkalinity may be produced by the ammoniacal decomposition of peptones, &c., and the same process occurs regularly in milk. *No indole* is produced in peptone-water. The *abortus* type produces H_2S; the *melitensis* type does not.

Antigenic structure. The agglutination-test is the best means we have of identification. Each species has two specific smooth-phase antigens (M and A,

Table XIV), present in each in different proportions. A serum made with a fresh strain of either species agglutinates that species to full titre, and the other to a lower titre. Long cultivated strains lose the M and A antigens, and show only a common rough

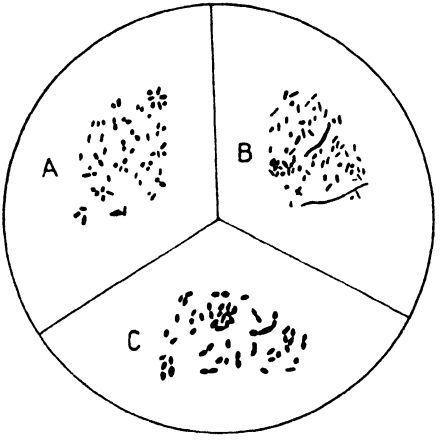

FIG. 16. *Brucella. Haemophilus*
A. *Brucella* (*melitensis* or *abortus*).
B. *Haemophilus influenzae.*
C. *Haemophilus pertussis.*

antigen. The so-called paramelitensis and para-abortus strains are in this state.

Pathogenicity; toxins. Just as *cattle* and *swine* are the reservoir for *Br. abortus* infection in man, so the *goat* stands in relation to Mediterranean fever. This animal suffers from a natural septicaemic infection with *Br. melitensis*, which is excreted in large numbers in its milk during and often long after an attack. *Br. abortus* infection occurs also in horses, dogs, sheep,

goats, and rats. Both *Brucellae* are experimentally pathogenic to guinea-pigs, rabbits, and monkeys. To monkeys and man *Br. melitensis* is the more virulent of the two. Thus Mediterranean fever is a more serious illness than 'abortus' fever, and *Br. melitensis* is the more dangerous to handle in the laboratory, where a number of accidental infections with pure cultures have been recorded.

TABLE XIV

Antigenic structure and variation of Brucella

Phase	*Thermo-agglutinability* (90° C. 60 *mins. saline suspension*)	*Antigens* *Melitensis*	*Abortus*
Smooth	0	M(· A)	A(· M)
Rough		R Paramelitensis or para-abortus	

Channels of infection. Both these fevers are mainly *milk-borne infections*; the Mediterranean variety being transmitted by goats' milk; 'abortus' fever by the milk of cows. In Europe, the U.S.A., and elsewhere, milking-herds are infected with *Br. abortus*, about 20 per cent. of the cows excreting the organism in their milk. This being so, it follows that the natural immunity of man must be very high. Apart from the consumption of milk, intimate contact with infected animals (including swine) is an occasional cause of infection. Moreover, it has been shown that herdsmen, veterinary surgeons, and milkers give an unduly high percentage of positive agglutination-tests against *Br. abortus*, which may be taken as evidence of *subinfection* (p. 9). Goat-herds are similarly exposed to infection and subinfection with *Br. melitensis*. The microbe may be cultivated from dung or dust in the neighbourhood of stables. Although human carriers

are not uncommon and may occasionally transmit infection by their urine, &c., they are not an important source of epidemic spread.

The disease in man. Mediterranean fever which is especially prevalent in Malta, but occurs in many countries with warm climates, is a septicaemia with inflammatory enlargement of the spleen, liver, and lymph-nodes, and often effusions into the serous cavities and joints. The fever comes and goes in undulations lasting 1 to 3 weeks.

The microbe is usually to be found in the blood, and is excreted intermittently in the urine. Isolated abscesses in bones and other parts are an occasional late result of spread through the blood. Though the mortality is seldom high (about 2 per cent.) the illness often lasts for many months with alternate remissions and exacerbations.

Undulant 'abortus' fever is of precisely the same nature, though generally milder and seldom fatal. It may be very hard to distinguish clinically from relapsing enteric fever. Infection during pregnancy may give rise to abortion.

The *toxins* of the Brucellae are so far as we know entirely intracellular. The production of agglutinating, opsonic, and complement-fixing antibodies follows the usual course during the illness, but the microbe is evidently able to resist destruction until a succession of relapses has raised the immunity mechanism to a high degree of efficiency (p. 235).

In Brucella infections a state of hypersensitiveness to the endotoxin of the organism is developed, as can be shown by the intracutaneous injection of 'melitin' or 'brucellin' preparations, analogous to tuberculin (p. 148). The test, however, cannot be relied on for diagnosis.

Active and passive immunization in the form of pro-

phylactic and therapeutic serums and vaccines are of doubtful efficacy.

Diagnosis. The *agglutination-test* of the patient's serum with killed suspensions of *Br. melitensis* or *abortus*, or both, according to the locality and the history of the case, usually reveals the nature of the infection. In both illnesses the serum agglutinates both suspensions; but the infecting organism usually shows the higher titre, if the test is properly performed. The titre ranges from 1 in 50 to 1 in several thousands according to the stage of the illness: whereas normal persons and enteric fever patients seldom give a reaction at 1 in 25.

Blood culture is successful in the great majority of cases in the early stages, and often throughout the illness. In 'abortus' fever, cultivation must be done in an atmosphere containing CO_2 (see p. 117). Aseptic spleen-puncture is sometimes performed when the blood-culture is unsuccessful, or when a post-mortem diagnosis without a full autopsy is desirable. The organism is identified by cultural and agglutination tests.

Cultivation from the urine, by plating on agar enriched with glucose, &c., is also useful. The colonies may take several days to develop.

Diagnosis in animals (goats, cows, &c.) is usually made by bacteriological examination of the milk; by injecting it into guinea-pigs; and by testing its agglutinating-powers on the microbe. Blood-culture and blood-agglutination tests may also be done.

HAEMOPHILUS *and similar organisms*

(*Catarrh, whooping-cough, conjunctivitis, soft sore*)

This is a rather mixed group of tiny Gram-negative, non-motile, non-sporing, unencapsulated rods, which require or prefer blood or its derivatives for their full

development in culture, and are mostly found in respiratory infections.

The most important species are (1) *Haemophilus influenzae* (Pfeiffer's bacillus) and (2) *Haemophilus pertussis* (Bordet-Gengou bacillus of whooping-cough). The group also includes *H. ducreyi* (Ducrey's bacillus) of 'soft sore' or chancroid, an uncommon venereal disease; and the 'Morax-Axenfeld bacillus' (*H. lacunatus*) of subacute conjuctivitis. Both these need or prefer blood-media, and are distinguishable from *H. pertussis* and *H. influenzae* by morphological cultural and serological tests.

Haemophilus influenzae

This organism occurs chiefly in catarrhal conditions of the air-passages, such as colds, influenza, chronic pharyngitis, and broncho-pneumonia. It can also be found in small numbers in many healthy throats. In spite of its name it can no longer claim to be the cause of influenza, which is due to a virus (p. 220).

Its most outstanding characters (p. 109, and Fig. 16) are its minuteness and its inability to multiply without accessory growth-factors derived from haemoglobin or certain vegetable tissues. In sputum and young cultures the average cell length may be no more than 0·5 μ, but longer rods and even long unsegmented threads are to be seen in most cultures. Some variant races, especially those originating from cases of 'influenzal' meningitis, consist chiefly of the filamentous form.

The accessory growth-promoting substance, which must be added to agar to obtain a growth of *H. influenzae* has been shown to consist of two fractions:

(1) The V factor, analogous to but not identical with vitamin C. This is destructible at 120° C., and obtainable from blood, vegetable cells, yeast, and bacteria.

(2) The X factor. A heat-resistant derivative of haemoglobin; but also present in certain animal and plant tissues (e.g. potato). The haematin in heated blood is active, whereas fresh haemoglobin is not.

The synthesis of the V factor by various bacteria is shown by the increase of growth of *H. influenzae* on platings near colonies of other bacteria. As regards the X substance, it seems to be a catalase which protects the microbe against the H_2O_2 formed during growth. One of the best media is that of Fildes, in which a clear peptic digest of blood, previously prepared in bulk, is added to agar as desired.

The colonies, which appear in 24 hours, are small, transparent, circular, and flat, reaching 3 mm. diameter on a good medium. Rough colonies with wrinkled surface and serrated edge are met with in old cultures.

Liquid media are not much used; but fairly profuse growth takes place in blood-broth if *good aeration* is arranged for by putting only small quantities of medium in each tube.

Biochemical actions. Various carbohydrates, when added to a suitable liquid medium, are acidified without gas production. Some races produce indole, others none.

Antigenically, the species is exceedingly complex; the varying combination of numerous diverse antigens in the different races makes effective serological classification impossible. It has, however, recently been claimed that the majority of strains isolated are commensal rough forms, and that the true pathogens form very smooth, mucoid colonies, are capsulated, and much more homogeneous antigenically. All this, however, needs confirmation.

Pathogenicity; toxins; lesions. H. influenzae is neither naturally nor experimentally virulent to domestic and laboratory animals; though if injected in large doses

it exerts a considerable endotoxic action. A febrile catarrhal infection can be induced in man and monkeys by spraying the throat with pure cultures; but neither the symptoms nor the blood-changes are characteristic of influenza. In the natural human infection, whether acting alone or in conjunction with pyogenic cocci or viruses, *H. influenzae* causes an acute catarrhal and suppurative inflammation, usually in the naso-pharynx, bronchial tree, and lungs. It occurs also as an occasional cause of otitis media, nasal sinusitis, urethritis, prostatitis, arthritis, meningitis, endocarditis, and other acutely inflammatory conditions, often in conjunction with other pyogenic bacteria.

'*Para-influenza bacilli*' is a provisional name for various incompletely investigated organisms, mostly from the human throat, which differ from *H. influenzae* chiefly in being able to grow without the X factor, though they cannot do without the V substance.

Haemophilus pertussis

(*Whooping-cough*)

This tiny ovoid, rod-shaped organism is found exclusively in the muco-purulent exudate of the upper air-passages in uncomplicated whooping-cough, and in the broncho-pneumonic lung-lesions which so often complicate the illness. It is seldom, if ever, to be found in healthy human throats, and it has never been isolated from animals or from the outside world.

The organism bears a general resemblance to *H. influenzae* (Table XI, p. 109), but is readily distinguishable by cultural tests.

Morphologically it is a rather stouter and *more definitely ovoid cell*. On primary isolation, it shows many diplo-forms like tiny pneumococci. In young cultures unsegmented filaments are seldom seen.

For cultivation a rich medium, preferably containing

fresh blood, is needed at first. The blood-potato-agar of Bordet, rich in animal and vegetable growth-promoting substances, is by far the best for primary isolation, but the organism will also develop on serum-agar, heated blood-agar, or serum-broth. After some generations on serum-media it can be induced to grow on simple agar and broth. Development is characteristically slow; two days are required for the appearance of a delicate transparent film or tiny, hardly visible colonies, but on the 3rd and 4th days the growth on Bordet's medium takes its characteristic form either as a whitish film with a dull metallic lustre like aluminium paint, or as *isolated colonies* which vary in size from pin-point to large pin-head, and have a *very characteristic sheen, standing up from the surface of the medium like half-pearls or droplets of mercury.* (Contrast *H. influenzae*, p. 123.)

The *biochemical actions* of the organism are difficult to determine and of a generally negative character. No fermentation of carbohydrates, no indole-production, and no liquefaction of gelatin.

Its *serological characters* and *action on animals* are connected in a very interesting way with its cultural peculiarities. Freshly isolated cultures, or cultures grown exclusively on blood-media, are toxic to guinea-pigs; an intraperitoneal dose of about 2 mg. of moist culture, alive or killed, being fatal in about 48 hours. On the contrary, old strains on egg or agar are generally non-toxic.

This change, which results from cultivation, is accompanied by a profound antigenic alteration. The main antigen of the freshly isolated culture is lost, and a series of other antigens takes its place. The change corresponds to the smooth-rough variation of other bacteria, though in *H. pertussis* the usual colony-differences are difficult to make out.

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Pathogenic action on man. The proof that *H. pertussis* is the true cause of whooping-cough, which is still disputed by a few authorities, rests on the following grounds:

(1) The organism is to be found in the great majority of cases of whooping-cough in the early stages, and there is good reason to believe that it could invariably be detected by sufficiently careful examination.

(2) It does not occur in healthy persons, nor in catarrhal states unconnected with whooping-cough (contrast *H. influenzae*, p. 122). Only two exceptions to this rule are on record, in spite of very numerous searches.

(3) A disease with all the symptoms of whooping-cough has been transmitted both to monkeys and man by means of pure cultures, and the microbe has been recovered from the lesions.

(4) In the majority of cases specific antibodies to *H. pertussis* can be detected in the blood-serum by the complement-fixation test at a late stage of the illness.

The lesion is a superficial inflammation of the tracheal and bronchial mucous membrane, with minute areas of necrosis and ulceration. A downward spread of the infection may give rise to peri-bronchiolar inflammation and patchy broncho-pneumonia in which other bacteria, especially *H. influenzae*, may take part. No general invasion of the body occurs, but various nervous and other symptoms suggest the absorption of endotoxin.

The simple uncomplicated disease is hardly ever fatal, though it often leaves prolonged debility in its train. But the pneumonic complications cause a very high mortality in the poorer sections of the population.

Diagnosis. This is best made by the '*cough-droplet*' method. Petri-dishes of Bordet's potato-blood medium are taken to the bedside, and the nurse or mother

exposes them for 5 or 10 seconds each in front of the patient's mouth during a natural attack of coughing. They are then returned to the laboratory, incubated for 3 days and examined with a hand lens for the characteristic 'mercury-droplet' colonies. A Gram-stained film for morphology completes the test. In the first two weeks of the cough, usually before the whoop has developed, the test is positive in at least 3 out of 4 cases. Later on the organisms become fewer and fewer, and after the 5th or 6th week they can never be isolated.

If the cough-droplet method cannot be used, plating out of sputum on Bordet's medium gives fair results.

Immunization. Danish and American authors have produced evidence that large doses of 'smooth' vaccine have a considerable protective action against whooping-cough; but a more complete statistical proof is required. There is also some evidence that the serum of convalescents has a short prophylactic effect. Treatment of the established disease with serums and vaccines is ineffective.

CHAPTER IX

CORYNEBACTERIUM (DIPHTHERIA); PFEIFFERELLA (GLANDERS AND MELIOIDOSIS)

CORYNEBACTERIUM

THE Corynebacteria are Gram-positive, non-motile, non-sporing, unencapsulated, aerobic rods of distinctive form. The genus comprises the important *C. diphtheriae*, a number of saprophytic 'diphtheroid bacilli' and a few species that cause disease in animals (*C. pseudotuberculosis murium* and *ovium*).

Corynebacterium diphtheriae

(Bacillus diphtheriae. Klebs 1883, Loeffler 1884)

This is a pure parasite, found only in the nose and throat of cases or carriers of diphtheria and occasionally in infected wounds. Its main characters are shown in Table XV, p. 145, and Fig. 17. Certain distinctive, though variable, morphological features should be noted, viz. (1) Irregularly swollen and club-shaped cells. (2) Uneven staining, giving a cross-banded appearance. (3) Metachromatic granules (p. 20), especially at the poles. (4) Fission at first incomplete, the two cells remaining attached at an angle. Stained preparations show a 'palisade-arrangement' reminiscent of Chinese writing.

The average size of the cells is from 2 to 4 μ by 0·3 to 0·8 μ. The characteristic granules are well shown by *Neisser's stain*, which consists of a very short exposure to a blue dye followed by rapid treatment with a brown or pink stain. The granules show up purplish-blue in a brown or pink-tinged protoplasm. An equally effective

and quicker method is to examine the bacteria in a wet film of Bie's acid methylene-blue stain. True branching (Y forms, p. 43), which was first demonstrated in this species, is to be seen in some cultures.

Cultivation. Good growth occurs in 24 hours on

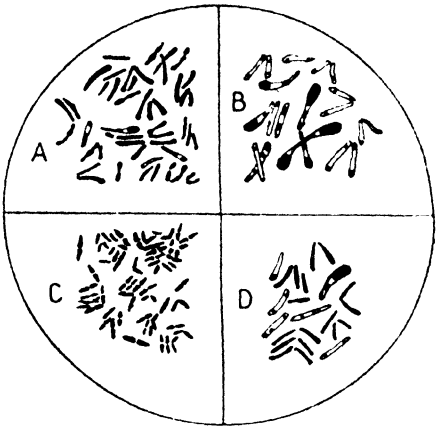

FIG. 17. *Corynebacterium diphtheriae* and Diphtheroids.

A. *C. diphtheriae.* B. *C. diphtheriae*, showing granules; also bands and clubs (involution-form). C. Diphtheroid (Hofmann type). D. Diphtheroid very like *C. diphtheriae.*

coagulated serum. On *agar* it is much poorer. In *broth* there is turbidity and deposit in 24 hours, and a thick surface growth or pellicle forms after a few days. The *colonies* on coagulated serum in 24 hours are small (1 mm.), greyish- or yellowish-white, smooth, moist, and circular. Older colonies are larger, yellower, and bossed in the centre like a poached egg. In some old stock cultures of rough colonies (p. 44) may be seen.

Biochemical action. In carbohydrate media enriched

with serum the non-gaseous fermentation of glucose, maltose, and dextrin, and the absence of action on lactose, saccharose, and mannitol, distinguish *C. diphtheriae* from the diphtheroids, none of which show precisely this range of activity.

Three *epidemiological types*, are now recognized: 'gravis', 'intermediate', and 'mitis'. The first two, and especially 'gravis', are the most virulent, while infection with 'mitis' is generally mild. All three produce the same toxin, but the more virulent types do not necessarily produce the most toxin, since virulence is also determined by resistance to phagocytosis, growth-rate *in vivo*, and other unknown factors. The three types can be distinguished by the characters of their growth on a blood-tellurite-agar medium and in broth, and by differences of fermenting power (e.g. 'gravis' ferments starch).

Antigens. Agglutination tests do not reveal any relationship between the types or between *C. diphtheriae* and the other Corynebacteria. The types themselves are not serologically uniform, since each comprises a number of antigenic sub-types.

Pathogenicity. The injection of cultures or culture-filtrates (toxin) into guinea-pigs and rabbits causes local necrotic inflammation and rapid death from intoxication. Congestion, cloudy swelling, and haemorrhages are found in the internal organs (liver, kidneys, and especially suprarenal glands,) and effusions occur in the serous cavities. Dogs, cats, horses, and various birds are moderately susceptible to experimental infection; rodents are highly resistant. The only animals known to contract infection by natural means are cows and fowls. The former are sometimes infected on the udder when milked by a human diphtheria-carrier; the latter occasionally suffer from true diphtheria of the throat, which must not be confused with the far

commoner 'fowl diphtheria' caused by an ultra-microscopic virus.

Diphtheria in man begins as an acute inflammation and ulceration of the faucial or nasal mucosa. A 'false membrane', consisting of necrosed tissue, fibrin, polymorphonuclear leucocytes and bacteria, forms on the raw surface. The absorption of toxin from the local lesion causes degeneration of the cells of parenchymatous organs, of nerve cells and muscle fibres, which results in organic disturbances and partial paralysis. The invasion is nearly always limited to the local lesion; the blood remains sterile, but contains toxin, which causes the general symptoms.

The toxin. This is a typical exotoxin (p. 49), which is obtained by filtering a ten-days' broth culture. It may be so powerful that one c.cm. will kill five hundred guinea-pigs (M.L.D. 0·002 c.cm.). The toxin slowly loses its strength on storing, and rapidly when heated to 60° C. or over. A similar detoxication is effected by the slow action of weak formalin (3 to 4 per cent. for a month). The resulting substance, called *toxoid* or anatoxin, is harmless to animals but retains its antigenic power and its combining affinity with antitoxin. The importance of this fact will be seen later (p. 49).

Antitoxin. The *antigenic* property of toxin is shown by its power of causing the production of antitoxin in animals. This process occurs also in the natural disease in man and is the main factor in recovery and subsequent immunity to infection. The classical experiments of von Behring (1890) proved that animals injected with virulent *C. diphtheriae*, or with its toxin, could be saved by the injection of the blood-serum of animals previously immunized by a course of toxin-injections.

For the treatment and prevention of human diphtheria antitoxic serum is made in horses (but see p. 242).

Its potency is measured in antitoxin-units, according to the following principles:—

The antitoxin-unit was originally defined by Ehrlich as the least quantity of serum which, when mixed with 100 minimal lethal doses of a particular, arbitrarily chosen batch of toxin and injected into guinea-pigs of 250 gm. weight, saves them from dying within 3 to 4 days. Thus, if the requisite amount is 0·01 c.cm. the serum is said to contain 100 units per c.cm. and so on.

In order to have a permanent standard-material with which to estimate a succession of batches of antitoxin, the toxin was dried and preserved *in vacuo*. But after a time it was found to be losing strength even under these conditions, and so another means of maintaining the standard had to be sought. The problem was eventually solved by drying and preserving a batch of antitoxic-serum which had been standardized against the original toxin. This was found to be perfectly stable over long periods. To standardize a new batch of antitoxin, some of the standard dried antitoxin was redissolved and a comparative series of protection-tests was done with (*a*) the new serum and (*b*) the standard serum, using any toxin of adequate strength. The potency of the new serum was thus found by direct comparison with the standard. When the original batch of standard serum was running out a new batch was standardized against it, dried and preserved as standard, and so on. This is the method in use at the present time.

The actual test of a new antitoxin is done by first finding how much toxin has to be added to one unit of standard antitoxin in order just to kill the animal. This is called the Lt (*Limes tötlich*) or often L† dose. Then this dose of toxin is mixed with graded quantities of the new antitoxin and injected into guinea-pigs. The greatest quantity of serum that can be added

without preventing death in 4 to 5 days is clearly the equivalent of one standard antitoxin unit.

Nowadays serum containing 800 to 1,000 units per c.cm. is commonly produced; and it can also be greatly concentrated by the chemical removal of a great part of the therapeutically useless proteins, which are responsible for the not infrequent 'serum sickness' following antitoxin treatment (p. 253).

Römer's intracutaneous method. New serums can also be standardized by making use of the local inflammatory and necrotic action of minute doses of toxin injected into the skin. The protective power of a new antitoxin is compared with that of the standard by mixing graded quantities of each with the toxin before injection. A considerable number of injections can be made in a single guinea-pig, which is a great economy. The method tends to replace the older one in all titrations except the final potency measurement of a serum for public use, for which the old method is considered safer.

Finally, there is the *in vitro* (test-tube) *flocculation test* (see p. 50) by which the potency of antitoxin can be measured with considerable precision. Its results do not, however, tally exactly with those of the animal tests, since a slightly different set of factors is involved.

Avidity. The theory of toxin-antitoxin-combination is considered elsewhere (p. 242). Some specimens of antitoxin combine so loosely with toxin that the compound dissociates on dilution, setting free some of the toxin. Such specimens are described as having a low 'avidity', and are nowadays rejected as unsuitable for therapeutic use.

Skin sensitivity. The Schick test. The skin of some adults and most children reacts to the intracutaneous injection of minute doses of diphtheria toxin (about 1/50 of the M.L.D. for a guinea-pig), by the formation

of a swollen reddish patch within 24 to 48 hours. This is called a positive Schick reaction (after its inventor), and may be taken, with certain reservations, as an index of susceptibility to diphtheria. The reaction is negative in about 80 per cent. of adults and in a fair number of children, and experience has shown that a Schick-negative individual is practically immune from diphtheria. It is a curious fact that about 80 per cent. of new-born babies give a negative reaction and are hardly ever attacked by diphtheria until their reaction changes to positive, as it usually does in a few weeks' time. A *positive reaction* appears to depend on the following factors:

(1) Natural sensitiveness of the skin to the toxin. This is absent in most infants, but develops with age (cf. p. 62). The skin of new-born rabbits is similarly insensitive to various toxins which cause a brisk reaction in the adult animal.

(2) Absence of appreciable antitoxin in the blood.

A negative reaction is given by (1) naturally insensitive infants and (2) individuals with a few hundredths of a unit or more of antitoxin per c.cm. in their serum.

Antitoxin is acquired as the result of an attack of diphtheria, or of a subinfection. Some new-born infants possess a little, having acquired it from their mother's blood and milk. The Schick-negative class, therefore, will include both infants who are naturally immune or have inherited some antitoxin, and adults who have acquired immunity either (*a*) by passing through an attack of diphtheria, of (*b*) by living long enough to be sufficiently exposed to subinfection. The longer the person has lived, the more likely his reaction is to be negative. The more he has lived in crowded districts, e.g. town-slums, the sooner will he acquire a negative reaction by subinfection.

A slight difficulty arises in practice owing to the 'pseudo-reaction' caused in some individuals by the irritating non-specific ingredients of the toxin-solution. To avoid mistakes on this count a control test is usually done on another part of the skin with heated toxin, which contains all the non-specific matter but no active toxin.

The practical importance of the test is that it enables us to determine whether a person is susceptible to diphtheria. If he is Schick-positive, i.e. susceptible, and is likely to be exposed to infection, some immunizing process (see below) may be desirable.

Channels of infection. Direct droplet-infection from cases of the disease or carriers of virulent *C. diphtheriae* is the commonest mode of spread. The only other important vehicle is milk, which becomes contaminated by a carrier or from diphtheritic ulcers on the cow's udder.

Diagnosis. This depends on identifying *C. diphtheriae* in cultures made from swabs applied to the lesions in the throat or nose. If membrane is present, a fragment may be removed with forceps. The swab or piece of membrane (previously washed) is streaked on the surface of coagulated serum, which is incubated for a night. Dry films are made from the mixed growth and also from the material on the swab, and are stained both with Loeffler's methylene blue and by Neisser's method, to show the metachromatic granules. The presence of characteristic bacteria establishes a high degree of probability that the case is one of diphtheria; and if the clinical symptoms agree, it can be taken as diagnostic. But in doubtful cases, in carriers, and in swabs from the nose, where diphtheroid bacilli are especially common, a pure culture should be obtained by plating out, and the fermentation-reactions should be investigated. If the organism

ferments glucose and is negative in saccharose it is presumably *C. diphtheriae;* and all that remains is to prove it virulent. For the virulence test 0·2 c.cm. of a fairly dense suspension is injected intracutaneously into two guinea-pigs, one of which has been immunized the day before by the injection of some 500 units of antitoxin. Virulent cultures cause an acute local inflammation, with or without necrosis, in the unprotected, but not in the immunized animal.

Carriers. Both convalescents and healthy persons who have been in contact with cases, may harbour virulent *C. diphtheriae.* Chronic carriers are, with few exceptions, Schick-negative. Their antitoxin protects them from invasion by their own microbe. Carriers of avirulent strains are harmless, but an attempt should be made to cure persistent virulent carriers by measures such as the surgical correction of abnormalities of the nose and throat.

Protective inoculation. (*a*) *Passive immunization.* The resistance of individuals or small communities to diphtheria can be greatly increased in an emergency by the prophylactic injection of antitoxin. But this passive immunity lasts only a week or two, since the antitoxin is soon destroyed or excreted. So long, however, as there is enough circulating in the blood, it aids the destruction of invading organisms by guarding the phagocytes from the paralysing effects of the toxin.

(*b*) *Active immunity.* When immediate protection is not especially needed it is much better to stimulate the individual to produce his own antitoxin. This can be done by injecting several small doses of toxin-antigen in the form either of raw toxin partly neutralized with antitoxin, or of formalin-toxoid. The former is not without danger, since too much toxin is sometimes liberated from the mixture by dissociation. Formalin-toxoid, which is non-toxic but strongly anti-

genic, is, therefore, preferable; precipitation with potash-alum prolongs its antigenic action on the tissues. Schick-negative individuals, being already protected by the antitoxin in their blood, do not need immunization. For those with positive reactions a course of three injections at weekly intervals is sufficient in 90 per cent. of cases to give a long-lasting protection, which reaches its maximum in 2 to 3 months from the first dose. Toxoid causes severe local reactions in a few individuals, but these can be detected by the 'Moloney test', i.e. intracutaneous injection of a small dose of toxoid at the time of the Schick-test.

An example of the efficacy of active immunization may be quoted from America. A group of 200,000 children was taken; half were Schick-tested, and the positives actively immunized, the other half being watched as controls. During the subsequent period of observation five times more attacks of diphtheria occurred among the controls than among the immunized, and all those among the latter were mild.

Antitoxin-treatment of human diphtheria. After the introduction of antitoxin in 1890 one or two small but well-controlled experiments, carried out in Danish and French hospitals, proved that it was as effective under clinical as under experimental conditions. Although the other statistics that have from time to time been adduced to prove its value are not always above criticism, after forty years of experience the medical opinion of the world is overwhelmingly in its favour.

Antitoxin should be given in approximately the following quantities directly the diagnosis is made. *Infants:* 2,000 to 10,000 units, according to the severity of the attack. *Children:* 3,000 to 20,000 units. *Adults:* 3,000 to 50,000. In severe cases with much intoxication the intravenous method is best, otherwise intramuscular injection gives sufficiently rapid relief. *Early*

treatment is vital. If the clinical diagnosis is reasonably certain, it is not advisable to wait for bacteriological confirmation, for the later in the disease the antitoxin is given the less is its effect. Intravenous injection is the ideal, since the maximum concentration of antitoxin in the blood is immediately attained; but there is more risk of severe general reactions in this method than in those which allow of slower absorption. A certain proportion of human beings are hypersensitive to horse-serum, and the resulting 'serum-sickness' (p. 253) is intensified by any previous treatment with horse-serum, such as anti-tetanus inoculation (p. 169).

If administered early, antitoxin causes a rapid improvement of the general condition and accelerates the healing of the throat-lesions. Complications are much rarer than in untreated cases. The paralysis which is often seen after serum-treatment is due to the damage already done to the nerve elements before treatment began.

Diphtheroid bacteria

A number of corynebacteria resembling *C. diphtheriae* in their chief morphological characters are found in various parts of the human and animal body (p. 266), e.g. *C. hofmannii* (Fig. 17), a non-fermenting skin-commensal which grows profusely on agar, and *C. acnes*, probably the joint-cause, with *Staph. aureus*, of acne vulgaris. The characters of these two have been worked out, whereas those of the rest of the non-pathogenic diphtheroids, including the conjunctival commensal '*C. xerosis*', have not.

PFEIFFERELLA

(Glanders and Meliodosis)

This small group consists of rather slender, Gram-negative, non-motile, non-sporing, unencapsulated,

aerobic rods, which produce very severe granulomatous infections of man and animals.[1]

Pfeifferella mallei

(Bacillus mallei; the glanders bacillus)

This organism is a strict parasite found only in lesions of horses and men. The average cell is a slender

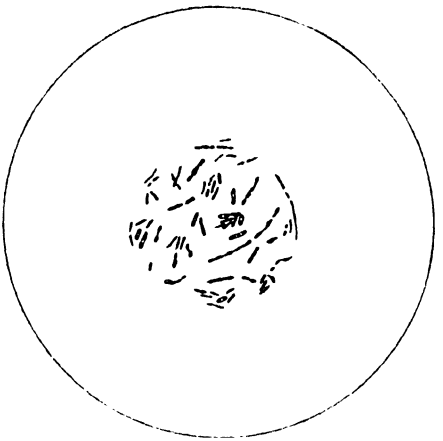

FIG. 18. *Pfeifferella mallei.*

straight rod about 0·4 μ wide and 1·5–3 μ long; but longer filaments are not uncommon. Its chief characters are shown in Table XV, p. 145, and Fig. 18.

Cultivation. On *agar* the growth is moist, slimy, and translucent, and becomes yellowish or brownish with

[1] The name of the genus should be *Loefflerella*, from Loeffler the discoverer of the glanders bacillus (1886). The title *Pfeifferella* is due to a clerical error in the report of the Committee of American bacteriologists who proposed this system of nomenclature.

primary lesion is on the skin it causes cylindrical swellings of the lymphatics (farcy-pipes) and enlargement of the nodes (farcy-buds). Man is generally infected by direct contact with diseased horses.

The typical lesion is intermediate between a granuloma and an abscess. It consists of a dense aggregation of leucocytes, mostly polymorphonuclear, often with some suppurative liquefaction in the centre. The bacteria are generally confined to the lesions and can seldom be found in the blood.

Toxins. Mallein. There is no evidence of exotoxin in filtrates of young cultures. An endotoxic product (Mallein) is prepared from old, autolysing cultures and used in horses as a skin test for the hypersensitiveness to which the infection gives rise (cf. tuberculin, p. 148). Glandrous horses react with a patch of inflammatory swelling, whereas healthy beasts are uniformly negative. This test, followed by the slaughtering of infected animals, has been largely responsible for the reduction of the incidence of horse-glanders in Great Britain from about 2,000 per annum before 1910 to less than 20 per annum since 1920.

Diagnosis in man. The purulent discharge from an ulcer is examined microscopically and cultivated by successive streaks on coagulated serum or potato. In favourable cases colonies of the bacillus will appear in two days at 37° C. At the same time one or more guinea-pigs must be injected intraperitoneally.

Pfeifferella whitmori

(*Melioidosis*).

A disease very like glanders, which occurs in human beings, cats, dogs, and rodents in certain parts of India, is caused by an organism resembling *Pf. mallei* in its chief characters, including the characteristic growth on potato.

A close relationship between the two is also demonstrable by cross-agglutination-tests.

Pfeifferella whitmori, however, differs from the glanders bacillus in the following respects. It is *motile*, grows more rapidly and abundantly, and often forms a gelatinous capsule. It also liquefies gelatin and produces acid from a number of carbohydrates in addition to glucose.

Melioidosis appears to be primarily a disease of rodents, which are also the most susceptible of all animals to experimental infection.

CHAPTER X

MYCOBACTERIUM. ACTINOMYCES

MYCOBACTERIUM

(*Tuberculosis; leprosy*)

THIS genus of *acid-fast*, non-motile, non-sporing, unencapsulated, slow-growing, aerobic rods, contains the important pathogenic species, *Myc. tuberculosis* and *Myc. leprae* which cause chronic granulomatous lesions in man and animals; also a number of saprophytic species which are found on grasses, in butter and manure (e.g. *Myc. phlei*) and on the genital and other mucous membranes of man (*Myc. smegmatis*). Finally, a species known as *Myc. paratuberculosis* or 'Johne's bacillus' causes a chronic enteritis of cattle and may be mistaken for the true tubercle bacillus in the microscopical examination of milk.

Mycobacterium tuberculosis

(Bacillus tuberculosis. Koch, 1882)

The species contains four varieties or types, each adapted to a different range of animal hosts; viz. the human, bovine, avain, and piscine or reptilian types. Certain differences of growth-habit and pathogenicity serve to distinguish these from one another (Table XVI). Only the first two types play any considerable part in human pathology, though the avian variety is said to have been found in a few cases of mild, atypical tuberculosis in foreign countries.

Human and bovine types. The human type is found chiefly in adult man, the bovine type in cattle and children (p. 150). Both occasionally infect other mammals.

Morphology (Fig. 19). The individual cells are rather slender, from 1 to 4 μ long and 0·3 to 0·4 μ broad, and often show banding or beading in stained preparations.

Staining. Simple stains give poor results. Ziehl-Neelsen's method (p. 17) is much the best. In young

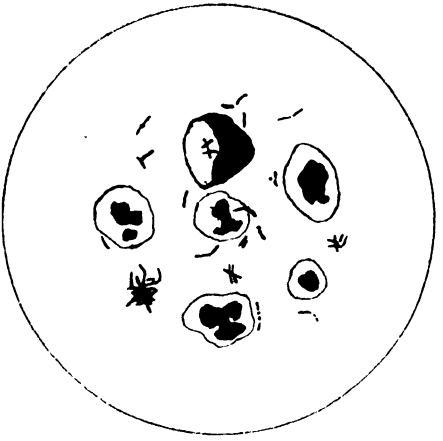

FIG. 19. *Mycobacterium tuberculosis* (in sputum, with degenerate leucocytes)

cultures a number of non-acid-fast cells are usually seen. It is often stated that alcohol will decolorize *Myc. smegmatis* but not *Myc. tuberculosis.* The distinction, however, is too slight and irregular to serve as a differential test.

Variation. Elongated and occasional branched forms (p. 31) are seen in lesions and cultures. Small non-acid-fast spheroidal bodies (Much's granules), stainable by a modified Gram's method, have been described as present in tissues, caseous matter, &c., but there is no

TABLE XV

SPECIES	MORPHOLOGY				STAINING		BIOCHEMICAL			
	Form	*Motility*	*Spores*	*Capsules*	*Gram*	*Acid-fast*	*Oxygen*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Mycobacterium tuberculosis	Small rods	0	0	0	+	+	AER	0	0	Egg; serum; glycerine in broth, agar, or potato 2 to 3 weeks required
Actinomyces bovis	Mycelium; branching filaments and rods	0	0	0	+	0	AN	0	0	Glycerine; glucose; blood or serum in agar or broth 3–4 days required
Corynebacterium diphtheriae	Clubbed and banded rods, medium size	0	0	0	+	0	AER	0	0	Coagulated serum, broth, &c. Glucose A Saccharose O
Pfeifferella mallei	Small thinnish rods	0	0	0	0	0	AER	0	A, slow C	24 hours required Ordinary media 2 days required A in glucose only

A = Acid; C = Clot; AER = Aerobic; AN = Anaerobic; 0 = None, or No change.

convincing evidence that they are alive or virulent. An ultramicroscopic (invisible), filterable form (p. 207) has been alleged to exist, but here the evidence is even less convincing.

Cultivation. No growth occurs on agar, gelatin, or broth, unless the medium is enriched with glycerine or blood. Egg-media are excellent; also coagulated serum and potato soaked in glycerine. In flasks of glycerine broth, the organism grows slowly in a spreading film on the surface.

Colonies. On solid media the colonies take 2 to 3 weeks to develop. They are greyish-white, or yellow, granular, dry and heaped up. The bovine type is smoother and moister than the human. Confluent films of growth have similar characters.

The resistance of the organism to chemical disinfectants is rather high. Thus, antiformin (which contains chlorine) kills it much more slowly than other bacteria, a fact which is utilized in the isolation of the organism from contaminated matter, such as sputum. Several hours' exposure to 5 per cent. phenol is necessary to sterilize tuberculous sputum or pus.

The *antigenic* constitution of the human and bovine types is the same, while each of the other types is different. The mycobacteria are troublesome to work with, owing to the difficulty of making good suspensions.

Action on animals. The subcutaneous injection of a very few living cells into a guinea-pig causes death in 3 to 12 weeks with an ulcerated nodule at the site of injection, enlargement of the regional lymph-nodes and lesions in the spleen, liver, and lungs. The organism can be seen microscopically in the lesions and isolated from them in pure culture. The bovine type is highly virulent to rabbits, which distinguishes it from the human and other types (Table XVI).

Tuberculosis in man. Although lesions may be found in almost any part of the body, the following sites are the most frequent; lungs, lymph-nodes, bones and joints, intestines and kidneys.

TABLE XVI

Some distinctive characters of the Mycobacteria

Species and Type	*Temperature for best growth*	*Growth in 4 weeks on solid media containing 5 per cent. glycerine*	*Experimental Pathogenicity*		*Natural pathogenicity*
			Rabbit	*Guinea-pig*	
Myc. tuberculosis. Human	37° C.	Profuse	(+)	+	Man, monkey &c.
,, Bovine	37° C.	Scanty	+	+	Cow, man
Avian	37°–45° C	Profuse and slimy	+	(+)	Birds (? man)
,, Piscine	22° C.	Profuse	0	0	Fish, reptiles
Myc. phlei, smegmatis, &c.	22° C. or 37° C	Luxuriant; highly pigmented; good growth in 24 hours	0	0	None

In the freshest lesions the bacteria are found inside large mononuclear reticulo-endothelial cells, the so-called *epithelioid cells*, some of which enlarge to giant-cells with a granular centre and a peripheral ring of nuclei. Around these a ring of lymphocytes accumulates, and the lesion is now termed an 'elementary tubercle' or 'Giant-cell system'. As the bacteria multiply and the lesion grows, necrosis of the central portions occurs, and at the same time young fibrous tissue is laid down outside the lesion. By the extension or fusion of several adjacent lesions a tiny visible mass, called a miliary *tubercle*, is formed. Further extension gives rise to a *tuberculous granuloma*, the necrotic centre of which softens into a whitish cheesy mass containing

dead and living tubercle bacilli. This is termed *caseation*. In chronic lesions *calcification* often results from the deposition of lime salts in the caseous matter.

Toxins, hypersensitiveness, and immunity. Tuberculin. Filtrates from young cultures in fluid media have little or no toxic action; i.e. no exotoxin is secreted. The cells, however, contain a specific material, called *tuberculin*, which is obtained by growing either a human or a bovine strain for 6 to 8 weeks in glycerine-broth, evaporating down at 100° C. to a tenth of the volume, and removing the bacteria by filtration. Various different tuberculins have been described, but the underlying principle is the same in all.

Tuberculin is hardly toxic at all to healthy infants, but for reasons given below, susceptibility increases with age. In most adults the subcutaneous injection of more than about a hundredth of a c.cm. provokes a painful local swelling with some general disturbance, but for a new-born baby the effective dose is about a hundred times more.

Now it has been shown experimentally that *animals suffering from tuberculosis are far more sensitive to tuberculin than normal animals.* Tuberculous human beings are especially sensitive; thus, a thousandth or even a ten-thousandth of a c.cm. is enough to cause an inflammatory swelling at the site of injection (local reaction), with fever and malaise (general reaction), and usually a 'focal' reaction around the lesion. The latter is indicated by redness or swelling, if the disease is superficial, as in lupus, or by an increase of pain or tenderness in deep lesions.

Koch's phenomenon. A curious experiment by Koch threw much light on hypersensitiveness in tuberculosis. The subcutaneous injection of virulent *Myc. tuberculosis* into a guinea-pig gives rise in about a fortnight to

a hard granuloma which gradually caseates and forms a discharging ulcer. If about 6 weeks after the first injection the animal is given a fresh dose of living culture subcutaneously, a totally different reaction takes place. Within 48 hours the skin at the site of injection becomes deeply inflamed and finally necrotic. The dead tissue, with the bacteria, is thrown off by ulceration, and the lesion heals up. Meanwhile the original infection runs its course.

Experiments have proved that the reaction of an infected animal begins to change about 10 days after the first infection, but that full hypersensitiveness takes some 40 days to develop. Thus an existing tuberculous focus protects a man or animal at least to a considerable degree from superadded tuberculous infection. Now it has been proved by careful post-mortem examinations that at least 90 per cent. of human beings are infected by *Myc. tuberculosis* in childhood or youth. The commonest lesion is a single focus localized in the lung, with a secondary infection of the lymph-nodes of the hilum. The lesions become ringed round with fibrous tissue and calcified, but may contain living bacilli for an indefinite time. It is believed that the disease takes this benign form when the dose of infection was single and small. The presence of latent lesions of this kind confers hypersensitiveness, as revealed by the tuberculin skin-test, and a consequent resistance to superinfection. A new and progressive infection may occur if the latent lesions heal or if the resistance is overcome by massive or repeated doses of bacteria. It seems that the latent foci seldom if ever cause active disease in later life. The long-drawn struggle of 'phthisis' is clearly due to a balance between invasion and resistance.

European races in general possess an effective 'infection-immunity' of the type described, but in certain

Eastern countries, where the microbe has not achieved a wide distribution, the individuals of all ages are so susceptible to infection that contact with Europeans may start devastating epidemics of tuberculosis. The same thing has happened in European populations whose resistance has been lowered by malnutrition.

Pathogenic differences of the human and bovine varieties. Both varieties are capable of causing fatal disease in man, but their frequency in the various types of tuberculosis is very different (Table XVII).

TABLE XVII

Type of Myc. tuberculosis in human lesions

Lesion	*Human type*	*Bovine type*
Lungs (Phthisis) . .	95–99 per cent.	1–5 per cent.
Abdominal organs, glands of neck, bones and joints	50–60 ,,	40–50 ,,

The bovine type is practically confined to children, who suffer more commonly from glandular or skeletal than from pulmonary infection. In pulmonary tuberculosis, whether juvenile or adult, the type is nearly always human. In lupus (tuberculosis of the skin) the two varieties are about equally represented, but the bacilli are, curiously enough, generally avirulent to animals.

Routes of infection. Without going into a controversial question, it is possible to deduce from the facts at our disposal that: (1) Children are infected either by contact with tuberculous adults whose sputum contains human tubercle bacilli, or by drinking milk from tuberculous cows. (2) Adults are practically always directly infected by human beings.

The chief vehicles of human-type infection are *droplets* coughed directly into eyes, nose, or mouth,

and *inhaled dust* containing dried particles of sputum, in which tubercle bacilli can survive for weeks. It has been shown that guinea-pigs can be infected with the washings from the walls of rooms inhabited by consumptives. The bacilli either reach the lung through the air-passages, or more probably are conveyed by phagocytic cells from the surface of the mucous membrane through the lymphoid follicles, and so reach the cervical or bronchial lymph-nodes. The passage of the bovine (or human) bacillus through the intestinal mucosa to the mesenteric glands occurs in a similar manner. It is significant to note that the waxy coat of *Myc. tuberculosis* enables it to survive 6 hours' exposure to the action of gastric juice, which destroys most bacteria in a few minutes.

Diagnosis. Pulmonary tuberculosis. Dry films of purulent flakes in the sputum are stained by Ziehl-Neelsen's method (p. 17), and searched for acid-fast bacteria of the right size and shape. In doubtful cases the test must be repeated several times, and the antiformin concentration method should be employed. In this method 1 part of the sputum is mixed with 2 or 3 parts of 20 per cent. antiformin, left for an hour and then centrifugalized. The strong alkali dissolves the viscous parts of the sputum, and most of the non-acid fast organisms are destroyed. Ziehl-Neelsen films are made with the deposit. If conclusive proof of the identity of the acid-fast organisms is required, or if none are found, cultivation should be attempted, and the most delicate test of all, injection into guinea-pigs, should be carried out. For the cultivation of sputum, which is always contaminated with various bacteria, the method of Löwenstein-Hohn gives very good results. The sputum is intimately mixed with five to ten times its volume of 10 per cent. H_2SO_4, left for 20 minutes and then centrifugalized. The deposit is

streaked on slopes of egg-medium; and some of it may be washed with saline and injected into guinea-pigs.

Bones, joints, and lymph-nodes. In Ziehl-Neelsen-stained films of the caseous matter from 'cold abscesses' the bacteria are scanty, but they can almost always be found if a yellow counter-stain (picric acid) is used. If none are found, about a c.cm. of the pus is injected into a guinea-pig, which will usually develop tuberculosis within 6 weeks. *Tissues*, either *post mortem* or removed during life for diagnosis (biopsy), are treated in the usual way and stained both with haematoxylin and eosin and by Ziehl-Neelsen's method. Since the tubercle bacilli are often very difficult to find, the diagnosis must often rest on the characteristic histological picture.

The tuberculin test for hypersensitiveness. A little tuberculin is introduced into the skin by making scratches through a drop placed on the front of the forearm (Pirquet's test) or is diluted about 1 in 1,000 and injected (0·1 c.cm.) into the layers of the skin (Mantoux's intracutaneous test). If, after 24–48 hours the skin shows a raised, red, tender swelling, about $\frac{1}{2}$ inch in diameter, the result is positive; if not, it is negative. A more or less quantitative measurement of hypersensitiveness can be made by injecting a number of serial dilutions into adjacent spots on the same arm.

Since, as we have seen, the great majority of healthy adults have, or have had, latent tuberculous lesions, their reactions to this test are nearly all positive (80 per cent. or more). The test, therefore, tells us nothing of the present activity of the disease. A negative reaction, however, especially if done quantitatively and repeated, can be taken as excluding tuberculosis. Infants are normally insensitive to tuberculin, so that a positive reaction indicates an active process. As the child gets older, the diagnostic value of the test for

active disease decreases proportionately. The tuberculin test is also widely used in cattle, with the object of excluding tuberculous animals from milking herds.

Serum-reactions in tuberculosis. Antibodies of all types are produced; agglutinins, precipitins, opsonins, and complement-fixing bodies, but they are, generally speaking, too feeble and inconstant to afford a reliable diagnostic test. The least uncertain is the *complement-fixation test* (p. 180) which gives, in experienced hands, up to 75 per cent. of positive reactions in well-established active cases; but it is not generally employed in routine work.

Specific immunization and therapy; B.C.G. The prophylactic treatment of infants and tuberculin-negative adults by the mouth with a *living vaccine* of a strain of *Myc. tuberculosis* attenuated by many years' growth on a medium containing bile is practised on a large scale in France and some other countries. Millions of children and adults have been treated with this 'Bacille Calmette-Guérin' or B.C.G., and it is claimed that the incidence and mortality of the disease have been greatly reduced. A serious accident, involving the death of 72 children at Lübeck, in Germany raised great doubt of the safety of the method, especially as experimental evidence was forthcoming that a throwback of the B.C.G. strain to virulence was possible. But after exhaustive legal and scientific inquiry the accident was attributed to the accidental substitution of a virulent strain for the harmless B.C.G.

The balance of evidence indicates that the vaccine has some protective value, but it is not enough to overcome the reasonable British prejudice against injections of living bacteria. It has been clearly proved that calves can be effectively immunized with B.C.G.; but the same can be said of Dreyer's formalin-killed, acetone-extracted 'diaplyte vaccine'.

Specific therapy. Vaccine-therapy in the usual sense (p. 248) cannot be practised, since killed tubercle bacilli are very irritating to the tissues, causing histologically typical, though entirely unprogressive lesions. Injections of tuberculin, however, have been widely used in the treatment of the disease, but the great tendency to spontaneous cure in tuberculosis makes it hard to judge the value of remedies, and therefore it remains uncertain whether tuberculin has any actual curative power. On the other hand, it is clear that much harm can come of the intense focal and general reaction caused by too large a dose.

Spahlinger claims both therapeutic and prophylactic value for his special antigens and antiserums, in the preparation of which great efforts are made to reproduce the natural environment of the microbe and to avoid chemical disruption of antigens. There is fairly good evidence of the prophylactic value of his vaccine in cattle, but no valid judgement can be given on the therapeutic aspect of his work.

Mycobacterium leprae

(Bacillus leprae. Hansen, 1874)

This organism is found only in the granulomatous lesions of human leprosy. A closely related species causes leprosy in rats, which does not seem to have any connexion with the human disease.

In its main morphological characters and staining reactions *Myc. leprae* closely resembles *Myc. tuberculosis.* In other respects our knowledge of it is greatly limited by the fact that, so far as we know, it *cannot be cultivated.* It is true that acid-fast organisms of various types have been grown from leprous lesions, but there is no conclusive evidence that any of them are the actual causative agent.

Action on animals. An extremely mild, local and

self-curing form of the disease can be given to monkeys (only) by inoculation with tissue emulsion containing the bacteria. Cultures of the supposed organism have no effect on any animal.

The *lesion of leprosy* is a progressive granulomatous infiltration of the deep layers of the skin. The microbes are to be seen in large numbers especially inside large mononuclear cells (lepra cells). In the *nodular* form, inflammatory spots appear on the skin of the face and extremities, and often on the mucous membranes of the mouth, nose, throat, and eyes. These thicken into nodules and often ulcerate, discharging serum and bacteria. There is little necrosis in the lesions, and never any caseation. In the *maculo-anaesthetic form* the peripheral nerves become diffusely infiltrated and the parts supplied by the nerves lose their sensibility. The skin atrophies and becomes secondarily inflamed. Various injuries and consequent deformities result from the anaesthesia.

Diagnosis. Dry films may be made of the exudation from ulcers of the skin, or with swabs from sores in the nose, and stained by Ziehl-Neelsen's method. The most certain method, however, is to cut sections of a fragment of tissue excised for the purpose. The characteristic intracellular distribution of the acid-fast organisms establishes the diagnosis.

Source and spread of infection. Leprosy is not highly infectious. It is generally contracted only after prolonged contact with cases of the nodular type. The incubation-time varies from a few weeks to several years. Infection probably enters through cuts or abrasions of the skin.

Prophylaxis and therapy. There is no effective form of treatment with specific serums or vaccines. Injections of chaulmoogra oil and its derivatives have a powerfully beneficial effect in early cases.

ACTINOMYCES

(Ray fungus)

The Ray fungi are filamentous, branching, mould-like organisms, generally classed among the Higher Bacteria (p. 14). Three types are pathogenic to man, viz. *Actinomyces bovis*, *Act. madurae*, and (rarely) certain acid-fast species. Several others cause granulomatous diseases in animals, and the rest live as saprophytes in the soil and on the grain of various grasses.

Actinomyces bovis

The chief characters of the organism are given in Table XV, p. 145. As its name suggests, the infection is commonest in cattle; its most frequent site being the mouth and tongue ('woody-tongue'). Small laboratory animals are not susceptible to infection.

Actinomycosis in man is relatively rare and generally takes the form of a chronic suppurating granulomatous infiltration of the skin of the face or neck, which may extend through the thoracic wall into the lung. The sites next in frequency are the vermiform appendix, mouth, and tonsil. General dissemination is rare. The case-mortality varies from 10 to 90 per cent., according to the accessibility of the site.

Histologically we see colonies or tufts of mycelium set in a soft mass of mainly polymorphonuclear leucocytes, surrounded by a zone of mononuclear cells, fibroblasts, and fibrous tissue (Fig. 20). In cattle the fungoid colonies are generally fringed with a row of club-like terminal swellings of the filaments, which give them a very characteristic appearance; but in the human tissues the clubs may be very difficult to make out. Their function is not known, but it is thought that they represent a defence-reaction on the

part of the parasite against the phagocytic and bacteriolytic action of the tissues, analogous, perhaps, to the functional capsule-formation of various bacteria (p. 24).

Channels of infection. It used to be taught that infection was due to punctures by sharp fragments of

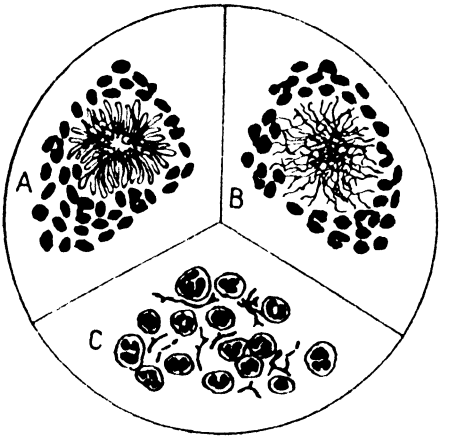

FIG. 20. *Actinomyces bovis*

A. Colony in tissues, showing clubs. Surrounding objects = cell-nuclei.
B. Mycellal mass in tissues. Surrounding objects = cell-nuclei.
C. Mycelial fragments in pus. Spheroidal bodies = leucocytes.

straw in stables, but we now know that the ray-fungi found on straw are saprophytes. Transmission is probably by discharges and droplets.

Diagnosis. The pus from the lesions contains grey or yellowish granules, often gritty from calcification. When crushed on a slide and Gram-stained they are found to consist of Gram-positive tangled filaments and shorter rods. In lung-infections similar grains are found in the sputum. *Cultures* (anaerobic) should be

made by smearing the pus on blood- or glucose-agar. The slowly developing *colonies* are small, circular, greyish-white, and soft. In glucose or serum-broth clumps of tangled filaments develop and sink to the bottom in a white mulberry-like mass. *Sections* of tissues, stained by Gram or various special methods show the picture described above.

Actinomyces madurae is one of the causes of 'Madura foot', a chronic granulomatous suppuration of the human lower extremity, occurring in India, S. Europe, and America. The organism differs from *Act. bovis* in being aerobic and forming dry, tough, heaped-up colonies. On potato the growth turns pink after a time.

Related organisms. Various other species of higher bacteria cause granulomatous diseases of animals; e.g. the aerobic, acid-fast *Act. farcinicus* which causes cattle-farcy (*Farcin-de-bœuf*), and a group of unbranched, filamentous species called *Actinobacillus*. Finally, a similar organism, *Erysipelothrix*, gives rise to a spreading erysipelatous inflammation of the skin of swine.

The saprophytic species of *Actinomyces*, e.g. *Act. graminis*, are distinguishable from *Act. bovis*, amongst other things, by their ability to grow at low temperatures (20° C.) and by the rapidity, profusion, and deep pigmentation of the growth.

CHAPTER XI

THE SPORE-BEARING RODS: BACILLUS, CLOSTRIDIUM

BACILLUS

(*Anthrax*)

THE genus *Bacillus* consists of large, aerobic, spore-bearing, mostly Gram-positive rods. A fairly constant feature is that the spore does not appreciably bulge out the contour of the containing cell, whereas in the genus *Clostridium* a definite bulge is the rule. Only one species, *Bac. anthracis*, is pathogenic. The remainder are common saprophytes of the soil and air, and interest us only as 'contaminations' of cultures, food, &c., e.g. *Bac. subtilis* (the hay bacillus), *anthracoides*, *megatherium*, *mycoides*, and *mesentericus vulgatus*.

Bacillus anthracis

(Davaine, 1863)

Bacillus anthracis (Table XVIII and Fig. 21) is one of the largest of the pathogenic bacteria. The average cell measures 5μ or more by about 1 to 1·2μ. In the tissues and body fluids the organisms are mostly single or in pairs, but in cultures on solid media they develop in long segmented chains or unsegmented filaments which interlace in wavy bundles, giving the colonies or film of growth a rough, felted appearance like tangled hair. Growth on ordinary media is rapid and profuse. In *gelatin stab-cultures* it is described as spreading out from the needle-track in the form of an inverted fir-tree; liquefaction sets in later, spreading downwards from the surface in the shape of a cup. In *broth* the bacillus grows in fine, thready flocculeS,

which settle to the bottom of the tube, leaving little or no turbidity.

Capsules, which are constantly present in the living body, are not formed on agar or in broth; but they may often be seen on media containing blood or serum.

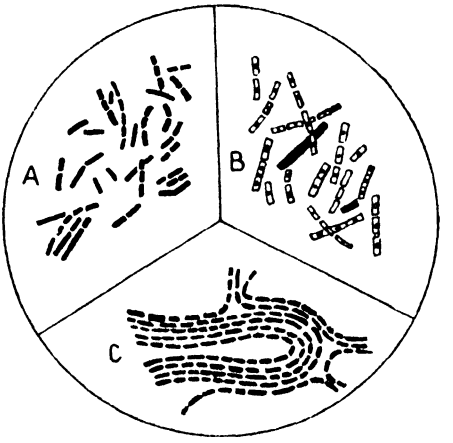

FIG. 21. *Bacillus anthracis*
A. Young culture; non-sporing.
B. Spore-bearing older culture.
C. Looped strand of growth at the edge of a colony.

Sporulation, on the contrary, does not occur in the living body, but takes place in old cultures of all types.

Variation is seen far more commonly under cultivation than in nature. Some races may produce a large number of grotesque involution forms; others completely lose the faculty of sporulation. We have mentioned elsewhere (p. 52) the loss of virulence that takes place when *Bac. anthracis* is cultivated at temperatures above its optimum.

TABLE XVIII

Sporing organisms

SPECIES	MORPHOLOGY				STAINING		BIOCHEMICAL			
	Form	*Motility*	*Spores*	*Capsule*	*Gram*	*Acid-fast*	*Oxygen*	*Liq. of Gelatin*	*Milk*	*Nutritional needs and actions*
Bacillus anthracis	Large rods	0	Equ.	(+)	+	0	AER	+ (slow)	C & P	Ordinary media
Clostridium tetani (Bacillus tetani)	Thin rods	+	Term	0	+	0	AN	+	0	,,
Clostridium welchii (Bacillus aerogenes capsulatus)	Stout shortish rods.	0	Subt.	+	+	0	AN	+	AGC ++	,,
Clostridium septique (Bacillus oedematis maligni)	Fairly large rods	+	Subt.	0	+	0	AN	+	A(G)C	,,
Clostridium botulinum (Bacillus botulinus)	Large rods	+	Term. or Subt.	0	+	0	AN	+	Alk. P.	,,

+ = Positive; 0 = Negative; Equ. = Equatorial; Term. = Terminal; Subt. = Subterminal; AER = Aerobic; An = Anaerobic; A = Acid; G = Gas; P = Peptonized (digested); C = coagulated; ( ) = variable.

Biochemical actions and *serological reactions*. These are not much employed in practice. Several carbohydrates are acidified, without gas. No indole is produced. Precipitation and complement-fixation reactions with the serum of immunized animals show that the antigens of *B. anthracis* are partly specific, but there is also a strong group reaction with the saprophytic bacilli, due to a common polysaccharide body-component. This common factor clearly suggests a phylogenetic relationship.

Pathogenic action. Anthrax is primarily a septicaemic disease of cattle, sheep, and a few other animals. Man contracts it only through contact with animals or their products. Most mammals are susceptible to artificial infection, whereas the majority of birds and reptiles are immune. In rabbits, guinea-pigs, mice, &c., death occurs with septicaemia, enlarged spleen, and local bloody oedematous inflammation. It is significant that the natural resistance of pigeons and frogs can be overcome by chilling the former and by warming the latter (p. 235).

Toxins. Virulence is due to the specific antigenic protein in the surface layer or capsule, and the loss of virulence in growth at temperatures above 40° C. is due to a great reduction of capsulated cells. Although toxic substances are sometimes formed in broth cultures, they have not the properties of specific exotoxin. The destructive action of the microbe on the tissues is probably due partly to similar non-specific metabolites and partly to the toxic capsular antigen. *Bac. anthracis* does not produce haemolysin, whereas the saprophytic *Bac. megatherium* does—an illustration of the principle that haemolysins are not neccessarily connected with virulence (cf. *Vib. el Tor*, p. 106).

Anthrax in man. The primary lesion generally occurs on the exposed parts of the skin, such as the face;

but the throat (larynx), bronchi and alimentary tract are also sometimes primarily infected.

At the site of implantation an acute, oedematous, polymorphonuclear inflammation causes the formation of a vesicle containing cloudy fluid. The continued multiplication of the bacilli causes necrosis of the central area, while the spread of the infection produces new vesicles around the primary one. If early diagnosis and treatment are impossible owing to inaccessibility of the lesion, infected emboli from the local vessels carry the bacillus into the blood, where it multiplies and infects other organs. In this case enlargement of the spleen, bloody effusions into serous cavities and other manifestations of septicaemia herald a fatal termination. But in the majority of cutaneous infections early serum treatment, with or without excision of the pustule, prevents further extension, and about 90 per cent. of patients recover.

Channels of infection. Infection comes to man in some product of the cattle industry, especially in imported Asiatic hides. In England we used to be familiar with 'wool-sorters' disease' (pulmonary anthrax), which modern factory hygiene has made comparatively rare; but any trade which deals with hides, animal hair, bristles or carcasses carries the risk of infection. A few cases amongst the general public have been traced to the use of imported shaving-brushes. Finally, blood-sucking flies are a danger wherever anthrax is rife amongst the local cattle.

Recovery, immunity, antibodies. In animals survival from an attack confers a solid immunity to experimental injection even of large doses of virulent culture. The serum of such an animal, and equally that of animals which have been through a course of preventive inoculation, contains precipitins, agglutinins, and (probably opsonic) antibodies that protect rabbits

from experimental infection. No anti-endotoxic nor bactericidal property can be demonstrated.

An antiserum from immunized asses (Sclavo's serum), given intravenously at an early stage of the infection, has had a good deal of success in reducing the mortality in human cases; but its action is obscure, since laboratory tests may fail to demonstrate specific antibodies, and normal ox-serum seems to have a similar, though weaker, effect.

Active immunization with vaccines made from cultures attenuated by growth at 42° C., though it is too risky for use in man, has been widely practised in sheep and cattle, ever since Pasteur's dramatic immunizing experiment in 1881. But both its efficacy and safety vary greatly in different circumstances owing to irregularities of the attenuation-process. The danger may be reduced by simultaneous injection of antiserum. Recent evidence points to the capsular antigen as the main stimulant of immunity.

Diagnosis. The presence of characteristically large single or paired rods, and perhaps some filaments, in Gram-stained films of pustule-fluid may be taken as provisionally diagnostic. To confirm this, cultures from the pustule are made on agar and in broth with a fine swab. The final proof of the nature of the organism is obtained by injecting either a culture or the excised and pounded-up pustule into guinea-pigs.

The saprophytic bacilli mentioned at the beginning of this chapter are like *Bac. anthracis* in many ways, but are distinguished in some cases by *motility* (*Bac. subtilis*), and in others by a more feathery spreading of the colonies (*Bac. mycoides*), or by a highly wrinkled growth (*Bac. mesentericus vulgatus*). In case of doubt, however, virulence to animals is the final test. Although *Bac. anthracoides* in large doses may kill mice with septicaemia,

neither it nor the others can be confused with *Bac. anthracis*, which is lethal in minute quantities to small laboratory animals.

CLOSTRIDIUM

(*Tetanus; gas-gangrene; botulism*)

This group consists of anaerobic, spore-bearing, Gram-positive rods, which mostly inhabit the soil and the intestines of animals. Several species are very dangerous when they reach the tissues through wounds or abrasions. Thus *Cl. tetani* causes lockjaw (tetanus), *Cl. welchii*, *septique*, *oedematiens* and one or two others give rise to gas-gangrene. *Cl. botulinum*, growing in preserved foods, produces a powerful toxin which causes food-poisoning or botulism. Lastly we have a number of saprophytes (*Cl. sporogenes*, *Cl. butyricum*, &c.), which are distinguishable from the pathogenic species by their lack of virulence to animals.

Clostridium tetani

(Kitasato, 1889)

This organism is found in infected wounds, in cultivated soil, and in the intestines of herbivora. It is also sometimes to be found in the faeces of healthy men. In its sporing form it has a very characteristic drumstick appearance (Fig. 22), which, however, it unfortunately shares with at least one saprophytic species. The vegetative cell is a slender rod of variable length ($0{\cdot}5\mu \times 2$ to 5μ). Filaments are not infrequently seen in cultures. It is motile and is described as peritrichate.

Cultural and biochemical characters (Table XVIII, p. 161). On agar under strictly anaerobic conditions the growth, like that of *Proteus*, spreads rather quickly in a thin ragged-edged film, so that single colonies are difficult to observe and isolate. But a good growth of

separate colonies may be obtained in a deep shake-culture in agar or glucose-agar, inoculated with spores immediately after it has been sterilized in the autoclave and before the medium has absorbed any oxygen. There is some gas-formation, which splits up the

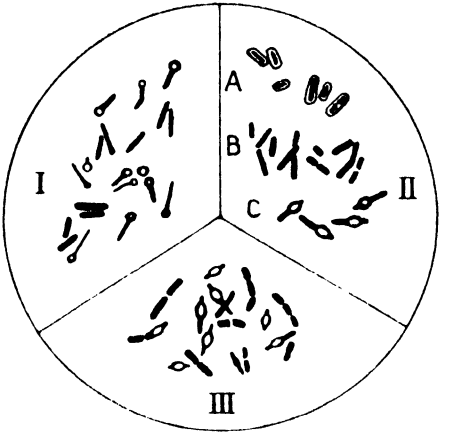

FIG. 22. *Clostridia*

I. *Cl. tetani*. Sporing (drumstick) and non-sporing forms.
II. *Cl. welchii*. A. Capsulated. B. Uncapsulated. C. Sporing.
III. *Cl. septique*. Sporing and non-sporing forms.

agar. In broth there is turbidity, some powdery deposit and a disgusting stench. *Minced meat, sterilized in water*, gives a good growth without anaerobic precautions (p. 36). Some species produce proteolytic enzymes which digest the meat, but *Cl. tetani* does not. *Indole* is formed in peptone water. No carbohydrates are fermented. On *blood-agar* a zone of *haemolysis* appears round the colonies.

Toxins and antigens. One of the salient characters

of the organism is its secretion of a potent *exotoxin*. Although there are at least seven serological varieties of the organism, all produce the same toxin. A good toxin (i.e. a filtered broth culture) will kill a mouse at a dose of a ten-thousandth of a cubic centimetre. Its physical and chemical properties are akin to those of diphtheria toxin (p. 131), but its effects are different, since it causes extreme irritability of the motor nerve-centres resulting in violent 'tetanic' spasms of the muscles. Like diphtheria toxin, it loses its toxicity on storing without an equivalent loss of antigenic potency, and the change can be accelerated by the addition of formalin (formol-toxoid).

Pathogenicity. Man and horses are susceptible to natural infection; guinea-pigs, rabbits, and mice may be killed in 2 or 3 days by subcutaneous injection of a broth culture. Birds and reptiles are insusceptible, owing to a lack of chemical affinity between their nervous tissue and the toxin.

Now it is a curious fact that *spores alone are incapable of causing tetanus*. If a culture is heated for half an hour at 65° C., both the toxin and the vegetative cells are destroyed, and the culture is now found to be harmless to animals, although the spores are intact. If, however, we add to the injected spores some agent that causes necrosis of the tissues, symptoms of tetanus will develop in due course. As necrotizing agent we may use sterile soil, calcium salts, living *Staph. aureus* or the toxin of *Cl. welchii*. But why is necrosis necessary? Recent researches have given the following answer. *In healthy tissues* the spores cannot germinate, owing to the relatively high oxidation-reduction potential (Eh, p. 36); and since without germination there is no production of toxin, no symptoms of tetanus develop. The majority of the spores are taken up by phagocytes and either digested at once

or dispersed through the tissues, where they may persist harmlessly for several months before dying out. *In damaged or necrotic tissues*, however, where the Eh is greatly reduced by lack of blood-supply, the spores germinate freely, toxin is produced, and an attack of tenanus results.

Thus, in the causation of tetanus in man, we can understand how the fouling of wounds with fertilized soil or road-dirt plays the double part of introducing spores (from manure, &c.) and of producing the tissue-damage necessary to allow germination.

Tetanus in man and animals. The *local lesion* is an ordinary acute suppuration, often of quite minor degree and usually containing a mixture of bacteria. The toxin diffuses out from the tetanus bacilli multiplying in necrotic foci, and, as careful experiments have shown, it is absorbed by the endings of the motor nerves, whence it passes up the axis-cylinders and thus reaches the anterior-horn cells. Its passage, under experimental conditions, may be blocked by division of the nerve-trunk, or by injecting antitoxin into it. The circulation of toxin in the blood-stream seems to play little part in the disease; for if an injection is made directly into a blood-vessel, and at the same time antitoxin is injected into the main nerve trunks of one limb, the resulting tetanus does not affect that limb, or appears in it much later than elsewhere. This indicates that even from the blood toxin must pass up the nerves to reach the central nervous system. It is thus easy to understand how it is that many tetanic attacks are local, i.e. affecting only one part of the body.

Defence-reaction. Tetanus toxin is a powerful stimulant to antitoxin-formation. If the infection is slight and the site not rich in motor nerves, the antibody-response may be sufficient in a few days to

neutralize the toxin *in situ* and prevent further absorption. But more commonly the response is too slow to prevent severe poisoning of the central nervous system; so that in cases not treated with antitoxic serum the fatality is in the region of 85 per cent.

Prophylaxis and therapy. Antitoxin is produced by immunizing horses with increasing doses of toxin, at first partly neutralized with antitoxin, or with formol-toxoid. Patients with a severe wound which may have been infected with cultivated soil or manure should be injected at once with about 3,000 units (I.U. or International Unit) of antitoxin, and the dose should be repeated weekly so long as the wound is septic, since the antitoxin is quickly excreted. The method of potency-estimation is similar to that of diphtheria antitoxin (p. 132). The unit adopted in the U.S.A. is double the strength of the European I.U.

The reduction of tetanus in the armies during the Great War as soon as antitetanus inoculation became general was extremely striking, as is shown in Table XIX.

TABLE XIX

Tetanus developing in Home Hospitals 1914–1916

Period		*Cases per* 1,000 *wounded*
1914	Sept.	15·9
	Oct.	31·8
	Nov.	1·7
	Dec.	0·8
1915	Average monthly	0·8
	Highest month	1·6
1916	Average monthly	1·1
	Highest month	2·8

Prophylactic serum treatment became general between Oct. and Nov. 1914.

Active immunization of soldiers with formol-toxoid (together with T.A.B. vaccine and diphtheria-toxoid) has recently been introduced.

The *treatment* of well-developed tetanus with antitoxin is, as might be expected, less satisfactory. In experimental guinea-pigs, if a given dose of antitoxin saves the animal from the effects of a lethal dose of toxin when the two are injected simultaneously, about two thousand times the quantity is required 24 hours later, when tetanic symptoms have set in. In man a dose of some 25,000 units is injected intrathecally at the earliest possible moment and a similar dose is given into the blood. Some days later the same quantity is injected subcutaneously to maintain the supply in the body by slow absorption. No statistics are available whereby to judge the therapeutic effect, but it is generally agreed to be beneficial in most cases.

Diagnosis by bacteriological methods is difficult and uncertain. Drumstick-sporing rods can sometimes be seen microscopically in the discharges from the wound, but it is not possible to be sure that they are *Cl. tetani* (see p. 165). This, however, can be proved by the *subcutaneous injection of mice or guinea-pigs* with material from the wound. One of the animals may simultaneously be protected by a dose of antitoxin, in which case tetanic symptoms develop only in the unprotected animal; the specific action of the serum proves that they are really due to *Cl. tetani*. Cultivation is too slow to afford a useful clinical diagnosis. The condensation water of a boiled blood-agar slope (or Fildes medium) is inoculated and the culture incubated anaerobically for one or two days. If a delicate film of growth (see p. 165) spreads upwards from the water, it can usually be proved to consist of tetanus bacilli.

The organisms of Gas-gangrene or Malignant oedema

The most important of the spore-bearing anaerobes found in this serious type of wound-infection are *Cl. welchii* and *Cl. septique* (or *Cl. oedematis maligni*). Their characters, as shown in Table XVIII, p. 161 and Fig. 22, are much alike, the chief primary distinctions being the *capsulation* of *Cl. welchii* and the *motility* of *Cl. septique*. The latter is generally the longer of the two: both are stout, but less so than *Bac. anthracis*. The subterminal position of the spore distinguishes them from *Cl. tetani*. Capsules are formed fairly constantly by *Cl. welchii* in the tissues, but cannot always be demonstrated in cultures. Both species *ferment* a number of carbohydrates with gas-production. The action of *Cl. welchii* on *litmus milk* is a very characteristic 'stormy fermentation', i.e. rapid acidification, coagulation, and gas-formation, which disrupts, inflates, and displaces the clot. Although *Cl. septique* produces all these effects, it produces them so much less quickly and violently that confusion seldom arises. This difference finds its parallel in the action of the two organisms on the tissues in gas-gangrene, for whereas emphysema (blowing out with gas) is a striking characteristic of *Cl. welchii* infection, it is much less pronounced with *Cl. septique*. Both have some proteolytic action on cooked meat and coagulated serum, but it is much weaker than that of some of the less important members of the group, e.g. the non-toxic *Cl. histolyticum*, which is often found in gas-gangrene in association with the more pathogenic species. This organism can digest even living tissues.

The pathogenic action of these organisms on animals such as guinea-pigs and rabbits depends on their

exotoxins. Washed cells and spores are usually quite harmless. The lesions are of the same type as those of the natural disease.

Gas-gangrene in man. In the wars of the pre-antiseptic era this disease was the scourge of military hospitals. Carried from wound to wound by the ignorance of doctors and nurses, it made the hospital more a menace than a refuge. Just as in tetanus, it is only in damaged and necrotic tissues that the spores can germinate; clean wounds, free from soil or other foreign matter, are seldom affected. The lesion is an acute, spreading, haemorrhagic, inflammatory, and gaseous oedema of the connective tissues. Toxins are produced, diffuse outwards, and extend the damage. Absorption into the blood-stream causes organic disturbances, fever, and degeneration of parenchymatous tissues. Invasion of the blood is not uncommon, and in fatal cases a gaseous decomposition of the body sets in with great rapidity. A similar decomposition is seen when an animal, after having been injected with a culture (especially of *Cl. welchii*), is killed and its body incubated overnight.

Apart from gas-gangrene there is evidence that 'welchii' toxin is one of the causes of intoxication in *intestinal obstruction*, and that the symptoms can be alleviated by injections of antitoxin.

Diagnosis. The mixed character of the infection, in which aerobic cocci and other bacteria share with the anaerobes in the production of the lesions, makes a quick bacteriological diagnosis impracticable in most cases. Treatment must therefore be based on the clinical signs and symptoms.

Antitoxin-therapy. There is good evidence that intravenous injection of horse-antitoxin alleviates the toxaemia and may save life. Probably the best practice is to give a mixture of antitoxins against *Cl.*

welchii, *septique*, and *oedematiens*, the last-named being the third of the toxic gas-gangrene organisms. The potency of the serums should be controlled by means of the League of Nations international standard serums. On the *prophylactic* side the scanty records suggest that a timely injection of mixed serum may prevent the development of the infection in badly wounded persons.

Clostridium botulinum

(*Meat-poisoning or botulism*)

This organism (Table XVIII, p. 161) is notable for its habit of growing in minced or preserved meat, such as ham, sausages (Lat. *botuli*), or canned foods, where it produces a toxin which has an evil effect on the consumer.

The cell is about the same size as *Bac. anthracis*, but motile, and its spores are terminal or subterminal. It has no very striking cultural peculiarities, and it is distinguishable from other anaerobes chiefly by the symptoms produced in animals by the *toxin* in culture filtrates. A large dose causes paralysis and death within 24 hours; smaller doses produce a more chronic paralysis chiefly localized in the injected limb. The action is chiefly on the end-plates of the nerves in muscles. Unfortunately, the species does not produce a single toxin, but there are at least three varieties, A, B, and C (? also D and E), each with a toxin of different antigenic constitution. Thus the *antitoxin*, which is of considerable value in the treatment of botulism, must be prepared against all three types of toxin.

The symptoms of botulism are entirely due to intoxication, for the microbe does not multiply in the body. The commonest are vomiting, thirst, paralysis of ocular and pharyngeal muscles, salivation and, in the worst

cases, delirium or coma and death. The mortality is often very high; nearly 70 per cent. in some outbreaks, but it may be as low as 20 per cent.

The *diagnosis*, made on clinical grounds, often needs to be confirmed, and the infection traced, by examination of the incriminated food, if available. A suspension of the material is made in saline and injected intraperitoneally into two mice, one of which has been previously treated with polyvalent antitoxin. Death of the unprotected mouse proves the toxicity of the food; survival of the other proves the nature of the toxin. Cultures may also be made from the food in cooked meat medium, incubated anaerobically for 10 days, filtered and tested on animals for toxicity.

CHAPTER XII

THE PATHOGENIC SPIROCHAETES

TREPONEMA (*Syphilis; Recurrent fever*)

LEPTOSPIRA (*Haemorrhagic Jaundice*)

SPIROCHAETES (Fig. 23) are spiral rods which differ from other bacteria in being *motile without flagella.*

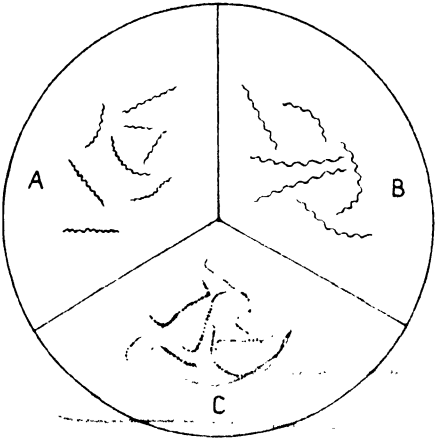

FIG. 23. *Spirochaetes*

A. *Treponema pallidum.*
B. *Treponema recurrentis.*
C. *Leptospira icterohaemorrhagiae.*

Their rapid corkscrew progression is caused by undulation of the body. In some species there is also a lashing movement of the body as a whole.

Ordinary bacterial dyes stain spirochaetes weakly or not at all. With the Giemsa or Leishman stains they take a pale blue or pinkish tint unlike the deep blue of

other bacteria. *Cultivation* is, in general, difficult, and can generally only be done in liquid or semi-liquid serum or blood-media in anaerobic or semi-anaerobic conditions. *Multiplication* is by tranverse fission. The extreme tenuity of many spirochaetes, whose diameter may be as little as 0·2μ, sometimes enables them to pass through bacteriological filters.

The majority of the spirochaetes live saprophytically in stagnant water and in the intestines of animals. Three species, however, are of great medical importance, viz. *Treponema pallidum* (syphilis), *Trep. recurrentis* (relapsing fever), and *Leptospira icterohaemorrhagiae* (haemorrhagic jaundice or Weil's disease).

Treponema pallidum

(Syn. Spirochaeta pallida. Schaudinn, 1905)

This is a thin spiral organism with tapered ends, some 0·25μ in breadth and from 3 to 12μ in length (Fig. 23). The spirals are close, regular, and rigid, with a wave-length of about 1μ. The spirochaete may be seen by dark ground illumination in scrapings from the primary sore or chancre of syphilis, and in silver-impregnated sections of infected tissues.

Cultivation is too difficult for routine work, and has in fact only occasionally succeeded. It is done anaerobically in a serum-water or serum-agar medium containing a piece of fresh rabbit-kidney, which lowers the Eh (p. 36) and provides accessory growth-factors. In practice the spirochaete is kept growing by injection into the testicles of rabbits, where it causes chronic inflammatory lesions; or into mice, in the tissues of which it survives and multiplies without causing any definite lesions or symptoms.

Pathogenicity. Man, apes, and rabbits are susceptible to natural or experimental infection. It is to be

noted that rabbits suffer from a natural infection (rabbit syphilis) with another species of spirochaete (*Trep. cuniculi*).

Syphilis in man. The initial lesion is usually on the external genitalia. It may also occur in the mouth or rectum, and occasionally on the fingers or other parts of the skin. Infection is almost invariably by direct sexual contact, and the organism is supposed to enter through insignificant cracks or abrasions, but there is good evidence that in rabbits it can penetrate intact preputial skin and mucous membranes. Rare instances of infection by contaminated drinking-vessels, tobacco-pipes, &c., undoubtedly occur, but the spirochaete is so feebly resistant to drying, light, &c., that only the immediate use of articles after contamination carries much risk.

The *primary chancre* which appears within 4 weeks from infection, begins as a small vesicle which soon ruptures, forming a tiny ulcer. This enlarges and its base hardens with leucocytic infiltration and fibrosis. Plasma cells and lymphocytes are more in evidence than polymorphonuclear leucocytes; and large macrophages, with occasionally some giant-cells, are also seen. The *regional lymph-nodes* become enlarged after a time, and though there are as yet no general symptoms, the spirochaete may already have passed into the blood-stream.

The *secondary stage* sets in some 6 to 12 weeks from the onset with fever, rash, and other symptoms of generalized infection.

In untreated cases, now very rare, this leads on to the *tertiary stage*, which lasts for months or years and consists of ulcerating and necrotic granulomatous lesions or gummata in all parts of the body; skin, internal organs, bones, and brain.

Finally, long after apparent recovery, a *para-*

syphilitic stage may set in, due to an inflammatory degeneration of the spinal cord and brain (tabes dorsalis or locomotor ataxia, and general paralysis of the insane).

In the secondary stage the whole body is invaded by the spirochaete. In the tertiary and parasyphilitic stages only scanty organisms are found in the gummata and central nervous system.

Latent infection. In some cases the organism, after causing an insignificant primary lesion, and no other symptoms, finds its way in small numbers into the central nervous system, where it lies latent for years, to manifest itself ultimately in the form of parasyphilitic paralysis. Persons thus infected may be entirely unaware of the fact; but it is likely that in the early stages they are infectious to others (i.e. are carriers).

Congenital syphilis. Syphilitic mothers may infect their unborn babies by the passage of spirochaetes through the placenta. There is no convincing evidence of paternal infection of the fertilized ovum.

Immunity. The human race has a poor natural resistance (p. 228) to *Trep. pallidum.* The specific defence-reaction is inadequate for the total elimination of the infection. No specific bacteriolysins, opsonins, or agglutinins are generally demonstrable in the blood. Inoculation experiments on rabbits and man have proved that the invasion of the spirochaete quickly produces a degree of resistance in the tissues, so that a secondary primary sore generally cannot be produced by a new dose of spirochaetes. But it seems that the species contains different races which do not protect against each other, so that a superinfection is possible if the second attack is by a race antigenically different from the first. The resistance to reinfection, which is evident in man and can be demon-

strated experimentally in rabbits, is conditioned, as in tuberculosis (p. 149), by the persistence of living spirochaetes in the body. It seems that as soon as all spirochaetes are eliminated, the refractory state of the tissues disappears. The immunity, in other words, is an *infection-immunity* in which the multiplication and spread of the organism are *kept in check*, but the tissues seldom, if ever, rid themselves completely of the spirochaete without outside assistance.

Chemotherapy. In the early stages of syphilis certain organic compounds of arsenic, e.g. salvarsan and its later substitutes, exercise a powerful anti-spirochaetal effect; but the action is not a direct destruction of the microbe, but probably a stimulation of phagocytosis by a kind of opsonic action of the drug on the microbe (p. 259).

Diagnosis. Scrapings from the *primary chancre* are examined by background illumination. The identification of *Trep. pallidum* is assisted by measurement of the spirals with a special ruled micrometer eyepiece. Other spirochaetes are usually present; of these *Trep. refringens* is much larger and thicker than *Trep. pallidum*, but there are various saprophytic species, both on the genitalia and in the mouth (e.g. *Trep. genitale* and *Trep. microdentium*), which are more easily confused, and considerable experience is needed for successful differentiation.

The serum-test for syphilis. Wassermann reaction. Flocculation tests.

In the early days of bacteriology, as soon as the diagnostic importance of the antigen-antibody reactions had been recognized, the attempt was made to apply them to the diagnosis of syphilis. The impossibility of cultivating the spirochaete ruled out the

agglutination-test, and attention was therefore turned to the *complement-fixation reaction*. To explain the rather complicated principles of this reaction a digression into the subject of *haemolysis* is necessary.

Haemolysis. If some red blood cells of an animal (e.g. a sheep) are injected into an animal of another species (e.g. a rabbit), the blood serum of the latter acquires, after some days, the property of haemolysing, i.e. dissolving the red corpuscles of the former species. Now this action has been shown to depend on two separate substances or properties in the serum. Thus, the lytic power of the serum weakens rapidly at room-temperature and entirely disappears in four or five days. Heating to 55° C. for half an hour similarly inactivates the serum; but in both cases it can be reactivated by adding a little fresh serum of any animal, even of the species from which the red-blood cells sprang. The substance or property which is inactivated by heating, &c., is called *complement*, because its action is complementary to that of the heat-resisting (thermostable) substance. This latter is known as *haemolytic antibody* or *haemolysin*. It has the property of combining specifically with the appropriate red corpuscles and 'sensitizing' them to the lytic action of complement. Other names for it are (*haemolytic*) *immune body*, and *amboceptor*, which indicates its power of combining simultaneously with corpuscles and complement (p. 242). Since amboceptor is very stable, strong haemolytic serum is generally prepared long in advance (e.g. by manufacturers) and stored for future use. Complement is provided on each occasion in the form of fresh normal guinea-pig serum; that animal having proved the best and most convenient for the purpose. The principle of the haemolytic reaction may be presented schematically thus:

Scheme of Haemolytic Reaction

Antigen	+	Amboceptor	+	Complement
(Sheep's red cells).		(Heated serum of rabbit immunized against sheep's red cells).		(Fresh guinea-pig serum).

Result: Lysis (laking) of the red cells.

Bacteriolysis. A reaction based on precisely the same principles occurs between *bacteria* and their specific antiserum. The bacteria take the place of the red cells; the amboceptor is an antibacterial antibody, and the complement is the same as before; thus:

Scheme of Bacteriolytic Reaction

Antigen	+	Amboceptor	+	Complement
(Bacterium)		(Heated serum of rabbit immunized with the bacterium).		(Fresh guinea-pig serum).

Result: Lysis (solution) of the bacteria.

In the course of either of these reactions the complement in the mixture (if not present in too great a quantity) is used up, so that if we set up a new haemolytic test and use, instead of fresh complement, the final fluid from an already completed haemolytic or bacteriolytic test, no complementary action will occur and therefore no haemolysis will take place. We shall thus have proved the *absence of complement* in the fluid; the complement having been *fixed* in the previous reaction by the sensitized red cells or bacteria. This brings us to the definition of *complement-fixation* as *the removal of complement by a combination of antigen with its specific antibody.*

Concerning the mechanism of complement fixation it is fairly clear that the first effect of the combination of antigens and antibodies is a mutual precipitation (p. 241). If the antigenic substance forms part of the surface of a cell, as it does in the haemolytic and bacteriolytic reactions, the precipitation or increased

colloidal aggregation occurs invisibly on that surface. If, however, the antigen is in colloidal solution, as it is in the case of toxins, a visible precipitate is formed. *In either case it is during the formation of precipitated aggregates of antigen and antibody that complement is absorbed and removed from the fluid.* It is, however, not only aggregates of this kind that absorb complement; almost any colloidal particles of a certain size will do so. This the specific element in the haemolytic and bacteriolytic reactions is not the fixation of the complement, but the combination of the antibody with its antigen, which leads to complement-fixation as a secondary result.

Now it is evident both in the haemolytic and the bacteriolytic reactions that if the antigen used does not specifically correspond with the amboceptor no combination or visible reaction will take place and therefore no complement will be fixed. The persistence of complement in the test-mixture can be demonstrated, as before, by using it (after centrifugalization) in place of fresh guinea-pig complement in a new haemolytic test. In this case the second reaction will be positive, i.e. haemolysis will occur. This, then, is a method that can be used either to identify an unknown antigen (e.g. a bacterium), by testing its complement-fixing power with a range of known antibodies; or, alternatively, to detect specific antibodies in a serum for diagnostic purposes, by testing it against a range of known antigens.

This system of reactions having proved useful for the diagnosis of certain infections, it was natural to explore its utility in syphilis. But in order to detect antispirochaetal antibodies in the blood a spirochaetal antigen was necessary. Cultures of the organism, however, were not available. How, then, was an antigen to be obtained? It occurred to Wassermann that

enough spirochaetal antigen could be extracted from the liver of a congenitally syphilitic foetus. Success was immediate; the foetal liver-extract, used as antigen and mixed with blood from a syphilitic patient gave complete complement fixation.

At first this seemed a triumph of deductive reasoning, but it soon proved to be merely a piece of luck. The observation was controlled by others with an extract of a normal human liver as 'antigen', and this, strangely enough, was found to work equally well. Further research showed that extracts of various normal organs, including cardiac muscle, could be substituted for the syphilitic liver without any loss of specificity, and it eventually became clear that in this case the *fixation of complement is not the result of a specific antigen-antibody combination*, but of a precipitation or flocculation of a lipoid constituent of the organ-extract by some unknown constituent, probably not a specific antibody, which is present in syphilitic blood in far higher concentration than in normal blood (Table XX).

In spite of this thoroughgoing falsification of its theoretical basis, the reaction remains one of the most specific and reliable of our serological tests. In practice large batches of extract (to which the term 'antigen' sticks tenaciously) are prepared by the alcoholic extraction of the muscle of calves' hearts after preliminary treatment with acetone. It is customary to add a small quantity of alcoholic solution of cholesterol, which increases the sensitiveness of the extract. For the test a small quantity is mixed with a measured volume of saline solution, which results in a cloudy suspension of microscopic particles of lipoid. It is the invisible aggregation of these particles under the action of syphilitic serum that effects the fixation of complement.

TABLE XX

Tests for Syphilis

(1) *The Wassermann reaction*

	Primary mixture	*Incubation*	*Addition to primary mixture*	*Result*
A. Diagnostic test	Heart-muscle extract (i.e. pseudo-antigen) Syphilitic serum (heated 55° C. 30 mins.) Complement (fresh guinea-pig-serum)	Complement fixed	Sheep's red cells sensitized with amboceptor (immune rabbit serum)	No haemolysis
B. Control test	Heart-muscle extract Normal serum (heated as above) Complement	Complement remains free	As above	Haemolysis

(2) *Flocculation reaction*

A. Diagnostic test	Heart-muscle extract Syphilitic serum	Visible flocculation
B. Control test	Heart-muscle extract Normal serum	No flocculation

The flocculation reaction. By suitably arranging the strength of the extract-suspension the *aggregation of the particles can be made visible*, and the whole reaction is vastly simplified. It is no longer a complement-fixation, and no haemolytic system is needed, for we are directly observing the process of which the complement and the rest were merely indicators. A great variety of methods have been based on this principle: the Sachs-Georgi reaction; Kahn's reaction;

the Sigma test of Dreyer and Ward, and numerous others.

The flocculation method is fundamentally simpler than the Wassermann, since it dispenses with the menagerie of animals required for the latter (guinea-pig, rabbit, and sheep), and is at least as accurate. Both types of reaction should be done *quantitatively*, by putting up a series of tubes with graded dilutions of the serum and constant quantities of the other reagents. For the Wassermann test preliminary titration of both amboceptor and complement is necessary to find out how much to use in the actual test.

Results of serum tests. The Wassermann and flocculation reactions give identical results about 95 times out of 100. Either may give an occasional false result, so that it is a common custom to do both with every serum.

The blood begins to give a positive reaction soon after the appearance of the primary chancre; in the secondary stage practically every case is positive, but later in the disease the average flocculating power of the serum sinks, with the result that between 15 and 20 per cent. of tertiary and parasyphilitic cases give a negative reaction.

Apart from syphilis, a positive reaction occurs regularly in Yaws, a tropical granulomatous infection of the skin with *Trep. pertenue*, which appears to be a variant of *Trep. pallidum*; and also in relapsing fever. The numerous reports of occasional reactions in certain other diseases are of doubtful validity.

The reaction usually becomes negative during treatment, but this is not a certain indication of cure, as it may revert to positive again later.

In addition to the blood-serum, the *cerebrospinal fluid* gives the reaction in a large proportion of cases of cerebrospinal and post-tertiary syphilis.

Treponema recurrentis

This spirochaete is the cause of relapsing fever, which is prevalent in eastern Europe, Asia, Africa, and America, and was not uncommon in England up to 1870. The different continents have their own names for the disease and for the causative spirochaetes, which are all variants of a single species.

Trep. recurrentis is a long organism with a spiral wave-length of about 2μ, roughly twice that of *Trep. pallidum* (Fig. 23, p. 175). During an attack of fever, which occurs in bouts of about 48 hours at intervals of from 2 to 21 days, the spirochaete can be seen in the blood by dark ground illumination, or may be stained in dried films by Giemsa's or Leishman's method. Rosette-forms, consisting of a number of spirochaetes adherent by one end, may be seen in the blood—apparently resulting from specific agglutination. *Cultivation* is easier than that of *Trep. pallidum*, and is done in similar media under anaerobic or semi-anaerobic conditions.

Transmission of infection is by the bite of ticks and lice, in the stomachs of which the spirochaete can be seen after an infected feed. The organism is pathogenic to man, monkeys, and various rodents.

Immunity. During the first attack specific antibodies which agglutinate, opsonize, and lyse the organism are formed in sufficient quantity to enable the reticulo-endothelial cells, especially of the spleen, to clear the blood; but a few spirochaetes lurking in the tissues escape destruction, and after a time begin to multiply again. The new strain is serologically altered and resistant to the antibodies evoked by the original strain. To eliminate this second wave of infection new antibodies have to be formed, and the same process may be repeated until the repertory of the spirochaete

is exhausted. The resulting immunity lasts for one or two years, after which reinfection is not uncommon.

Chemotherapy. Salvarsan and other arsenic compounds are as effective in relapsing fever as in syphilis.

Rat-bite fever. A human fever of the relapsing type which follows the bite of rats is caused by a minute spiral organism with flagella at each end. Since spirochaetes have no flagella this organism is classed as a *Spirillum* (*S. minus*). Some cases of this disease, however, are due to an *Actinomyces* (*Act. muris*).

Leptospira icterohaemorrhagiae

This spirochaete is primarily a parasite of rats and certain other rodents, in the kidneys of which it lives and multiplies without causing obvious illness. Infection reaches man through stagnant water or mud infected with rat's urine, and gives rise to a serious and sometimes fatal *haemorrhagic jaundice* (Weil's disease). In clean fresh water and sea water very small numbers of the organism can often be detected by cultural methods.

The spirochaete is very thin and so closely coiled that at first sight it looks like a chain of granules. One or both ends are usually bent in a hook, and the organism whirls and spins at a tremendous speed without much change of position. In heavy human infections it can often be seen in the blood by dark ground illumination; otherwise it is detected by the injection of the blood into guinea-pigs, which die in a few days with jaundice and haemorrhages into the subcutaneous tissue and serous cavities. The *agglutination-reaction* with the patient's serum and the formolized sediment of a fluid culture of the leptospira is also diagnostically valuable.

Cultivation is fairly easy under aerobic conditions in semi-solid agar mixed with fresh rabbit's blood. If

the material from which cultures are made is contaminated with other bacteria it may be purified by filtration through porcelain etc. (p. 207), since *Lept. icterohaemorrhagiae* will usually (though not always) pass through ordinary bacteriological filters.

A similar but non-pathogenic leptospira (*L. biflexa*) is common in dirty water and in dripping taps. A variety that inhabits the kidneys of wild mice appears to be intermediate in virulence between these saprophytes and the pathogenic species carried by rats. The agglutination reaction proves that *L. icterohaemorrhagiae* and *L. biflexa* are distinct species or varieties.

CHAPTER XIII

PROTOZOA; FUNGI: BACTERIA OF MINOR MEDICAL IMPORTANCE

PROTOZOA

(Malaria; sleeping sickness; kala-azar; amoebic dysentery

Plasmodium Malariae (&c.)

(Malaria)

THE name malaria expresses the primitive observation that the infection comes from bad air—the cold, damp air of nightfall in marshy places. Modern science has shown that danger is not in the air itself but in the mosquito flying in the air; and, further, not in the mosquito itself, but in the microbe it carries.

Malaria is an intermittent fever with anaemia due to the destruction of red corpuscles. Enlargement of the spleen is very common. The disease is widely prevalent in tropical climates and causes an immense deterioration of health and considerable loss of life. Three varieties are recognized (p. 192) according to the periodicity of the attacks and the average severity of the illness. In *benign tertian malaria* there is an average remission of 48 hours between the attacks; in the *benign quartan* type, 3 days. The *malignant* or *aestivo-autumnal* variety has a remission interval of from 24 to 48 hours (subtertian). These intervals correspond to the time required by the parasite for its *asexual developmental cycle* (schizogony) in the blood, the stages of which are shown in Fig. 24.

Life-cycle of the parasite. As soon as the organisms are introduced into the blood by a mosquito-bite they enter red blood-corpuscles and develop first into ring-

forms, then into half-mature and finally into mature plasmodia. While still in the cell the plasmodium, which at this stage is called a *schizont*, divides into a

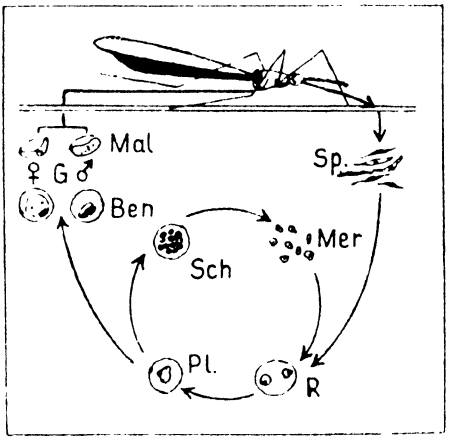

FIG. 24. Parasite of Malaria
Developmental Cycles

Inner circle. Schizogony. Mer. = Merozoites. R. = ring-forms in erythrocyte. Pl. = Plasmodium in erythrocyte. Sch. = Schizont in erythrocyte.
Outer circle. Sp. = Sporozoites. R. and Pl. as before. G. = Gametocytes. Mal. = Malignant type. Ben. = Benign type.

number of small *merozoites*. These are set free by the rupture of the cell and become attached to new erythrocytes in which (or possibly *on* which) they repeat the asexual cycle again and again.

After the disease has been running for a week or more, an occasional schizont, instead of developing into a new plasmodium, takes a different line and becomes a sexual cell, or gametocyte. In the benign forms these are hardly distinguishable from plasmodia,

though the female *macro-gametocytes* may sometimes be identified by their great size; but in malignant (aestivo-autumnal) malaria they assume the characteristic form of *crescents* (Fig. 24). No further development of these cells occurs in the human body, but if the blood is sucked by a mosquito, the gametocytes start a new *sexual cycle* of development (sporogony) in the stomach of the mosquito. The male or *microgametocyte* develops a vibratile flagellum which enables him to swim about in search of a mate. Allured by the chemical aroma of the female he effects a fusion and a *zygote* is formed. This cell, being motile and elongated, penetrates the muscle of the stomach wall, where it becomes a walled *sporocyst* inside which by repeated divisions a large number of long, thin cells, the *sporozoites*, develop in a radiate arrangement. When full-grown the sporocyst bursts, liberating the sporozoites into the body-cavity, whence a number find their way into the salivary-poison gland. It is the injection of these cells into the human blood with the mosquito's venom that starts the asexual cycle described above, the sporozoites entering the red cells and developing into adult plasmodia (schizonts). Owing to the fact that the sexual cycle occupies 10 or 12 days the mosquito is not infectious after an infected feed until this interval has elapsed.

Cultivation of the parasite is not possible in artificial media, but schizogony will proceed outside the body in defibrinated blood incubated anaerobically.

Channels of infection. Direct man to man infection does not occur in malaria. Animals are neither naturally nor experimentally susceptible. The disease has been transmitted to a human volunteer in England by mosquitoes fed on malarial blood in Italy and sent to England for the purpose.

Defence and immunity. The merozoites are taken up

TABLE XXI

The commoner protozoal infections of man

Disease	*Primary site of Disease*	*Parasite*	*Mode of transmission*	*Chemotherapy*
Malaria: (1) Benign tertian (2) Benign quartan (3) Malignant	Erythrocytes	(1) *Plasmodium vivax* (2) *Plasmodium malariae* (3) *Plasmodium falciparum*	Mosquito (*Anopheles*)	Quinine
Sleeping sickness	Blood Plasma	*Trypanosoma gambiense*	Blood-sucking fly (*Glossina palpalis*)	Arsenic compounds (Bayer, 205; Tryparsamide)
Rhodesian ditto	Do.	*T. rhodesiense*	Blood-sucking fly (*Glossina morsitans*)	Do.
Kala-azar	Vascular endothelial cells and plasma	*Leishmania donovani*	?The sand fly (*Phlebotomus argentipes*)	Antimony (Neostibosan, &c.)
Amoebic dysentery	Wall of large intestine	*Entamoeba histolytica*	Faecal (cyst) contamination of food and water	Ipecacuanha (Emetine)

and destroyed by phagocytes, but we have no evidence that specific antibodies play a part in defence. Immunity is gradually acquired by repeated attacks. If complete elimination of the parasite is achieved, susceptibility soon returns. Often, however, an apparent immunity is due to the persistence of the parasite, whose multiplication is kept in check (infection-immunity); in other words a balance is set up between parasite and host. The indigenous population of a malarial district generally has a considerable infection-immunity, and suffers far less frequently and severely from the acute illness than visitors from non-malarial regions.

Benign malaria is seldom directly fatal, and the fatality of even the malignant form is not very high. But repeated attacks of the latter may lead to a very serious condition known as *Blackwater fever*, in which the urine is dark with pigment originating from the intravascular destruction of red corpuscles. It is believed by some that the constant use of quinine plays a part in the genesis of the condition, but there is no conclusive evidence of this, nor of the participation of secondarily infecting organisms or special parasites.

Diagnosis. Dried films of the blood are stained by one of the Romanowsky methods (Leishman or Giemsa) and the corpuscles are searched for parasites. Quinine must be withheld previous to the blood-examination, since it greatly reduces the number of parasites.

Prevention. Since the *Anopheles* is the sole vehicle of infection, prophylaxis resolves itself mainly into an antimosquito campaign. The breeding-ground of the creature is in stagnant water and marshy places, so that drainage where possible is the first step. Next, the larvae which come to the surface to breathe can be

stifled by a thin layer of paraffin poured on the surface of ponds, water-butts, and the like. Individual protection by mosquito-nets at night is, of course, essential. In the Italian conquest of Abyssinia a daily dose of quinine completely protected the Italian soldiers from malaria.

The malarial treatment of parasyphilis. Syphilitic general paralysis is often greatly ameliorated by supervening febrile infections; and of these malaria has the most pronounced effect. This fact is exploited in the malaria-treatment of the disease by injections of infected blood, which cause prolonged remissions of the symptoms, though without permanent cure.

Trypanosoma

(*Sleeping-sickness, &c.*)

The trypanosomes (Fig. 25) are small, sinuous, wormlike, motile protozoa averaging about 25μ in length and 2–3μ in breadth. They have an *undulatory membrane* along one margin, terminating in a flagellum which projects forwards during movement. A *macronucleus* is to be seen near the centre of the body, and a tiny *micronucleus* or *kinetoplast* is situated at or near the origin of the undulating membrane at the rear end of the parasite.

Multiplication is by simple longitudinal division. No sexual cycle has been demonstrated, but it is thought that one may take place in the carrier insect (p. 192), since it does not become infective for about 20 days after an infected feed (cf. 'malaria', p. 191). In a Brazilian trypanosomiasis the organism (*T. Cruzi*) when multiplying in muscle-cells takes a form like Leishmania (Fig. 25. IV).

Cultivation of several species has been done aerobically on fresh, moist blood-agar.

Sleeping-sickness (*T. gambiense*) is especially prevalent on the west coast and various other parts of Africa. The characteristic nervous symptoms, lethargy and increasing drowsiness, are due to the pentration

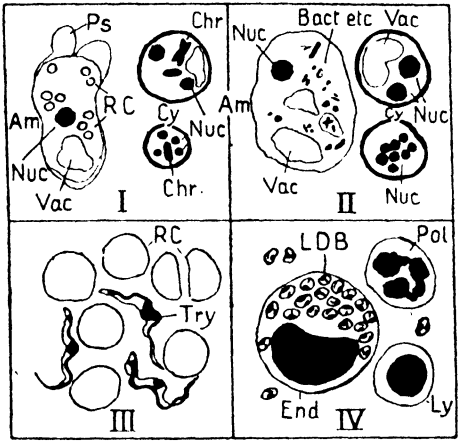

FIG. 25.

I. *Entamoeba histolytica.* II. *Entamoeba coli.*
III. *Trypanosoma.* IV. *Leishmania.*

I. *Entamoeba histolytica* (Am.) and two cysts (Cy.). R.C. = ingested red cells. Ps. = ectoplasmic pseudopod. Nuc. = Nuclei. Vac. = Vacuole. Chr. = Chromatoid bodies.
II. *Entamoeba coli* (Am.) and two cysts (Cy.). Bact. &c. = ingested bacteria and food.
III. *Trypanosoma gambiense* (Try.) in blood. R.C. = Red cells.
IV. *Leishmania donovani* (L.D.B.) in smear from spleen. End. = Endothelial cell. Pol. = polymorphonuclear cell. Ly. = Lymphocyte.

of the parasite from the blood into the cerebrospinal fluid, where it causes a progressive meningo-encephalitis. Enlargement of glands, anaemia, fever, and skin eruptions are some of the commoner signs and symptoms. Once developed, the disease is usually fatal, but

the infection does not by any means always progress to the point of causing recognizable symptoms. As in malaria, indigenous populations are widely infected, and acquire a tolerance for the parasite. It seems that cattle and certain wild mammals harbour the trypanosome in their blood without suffering harm from it, and act as reservoirs for human infection. Similar symptomless infections can be induced in guinea-pigs, rats, or mice by injection of blood from a case of sleeping-sickness. *Diagnosis*, *prevention*, &c., follow lines similar to those described for malaria.

Leishmania

(*Kala-azar*, &c.)

Kala-azar or *Leishmaniasis* is an irregular fever with anaemia and wasting, prevalent in India and various other parts of Asia and Africa. The liver and spleen are enlarged, and ulceration occurs in the intestines, and, less frequently, in the skin. The parasites, *L. donovani* or 'Leishman-Donovan bodies' (Fig. 25), are smallish spheroidal or ovoid cells of about 3μ diameter with two nuclear bodies, a macro- or tropho-nucleus and a smaller kinetoplast. In the body they are non-motile and have no undulating membrane or flagellum, but when spleen juice from a fatal case is mixed with sodium citrate solution and kept at about 20° C. some of the parasites develop into small, elongated, motile flagellated organisms like trypanosomes. The Leishmaniae are in fact flagellates closely related to the trypanosomes.

Distribution in the body. The parasites are found most easily in the spleen, and mainly inside phagocytic endothelial cells. They are, however, present in small numbers in the liver, marrow, mesenteric lymph-nodes and in the circulating blood. In ulcers of the intestine

or skin they may sometimes be seen in large numbers, but are often difficult to find.

In some cases of Leishmaniasis a chronic skin-ulceration (tropical ulcer) is the chief or only symptom. It is probable that different varieties of the parasite are involved in the different clinical types of the disease.

Mode of transmission. It has been shown that sand-flies can transmit the infection from human cases to animals (hamster), which suggests that they may be the chief vehicle of infection. No other mode of transmission, e.g. oral, has any experimental support.

The diagnosis may be made during life by spleen-puncture and microscopic examination of dry films stained by Leishman's method, but the procedure is risky. The examination of thick, stained blood-films gives a fair proportion of successes.

Entamoeba histolytica

(*Amoebic dysentery*)

The symptoms and course of amoebic dysentery are generally very much like those of bacillary dysentery (p. 93). Only very careful laboratory examinations can distinguish the two conditions.

The causative protozoon, *Entamoeba histolytica* (Fig. 25) is a large, irregularly spherical, ovoid, or pear-shaped cell of some 20 to 40μ diameter, consisting of ectoplasm and endoplasm and containing an eccentric nucleus which is not easy to see in unstained specimens. In fresh stools examined on a warm stage the parasite shows amoeboid movements, and ingested erythrocytes may be seen in its protoplasm.

In the earlier stages of the infection the amoeba multiplies by fission, and as the disease settles down to a more chronic condition *cysts* appear in the faeces.

These are smaller, spherical, thin-walled cells of about 10–15μ diameter, usually containing four nuclei and also some long deeply-staining 'chromatoid bodies' of unknown function. It is by means of these cysts that infection is spread, through the ingestion of contaminated food or water, and they may be excreted for years after recovery from the disease. Furthermore, cysts are often found in healthy persons (carriers) who have never suffered from clinical dysentery.

Experimental transmission by feeding to human volunteers has proved the pathogenicity of the amoeba. Kittens and dogs may also be infected.

Cultivation is not practicable in routine work and has only succeeded in a few instances. Media with an egg-basis have proved the most serviceable.

The primary lesion is an ulceration of the mucous membrane of the large intestine, preceded by oedematous inflammation without much aggregation of leucocytes. The deeper layers of the mucosa, the submucosa, and even the muscle are infiltrated by the amoebae, and the tissue-necrosis caused by their presence spreads laterally, so that the ulcer undermines the still intact membrane.

A common characteristic complication is *abscess of the liver*, which is really more a necrotic than a suppurative lesion. It contains a thick dark-coloured fluid and bits of detached dead tissue. Amoebae are not easy to find in the fluid but can be seen in histological sections of the abscess-walls. Abscesses also occur in the lungs, but much more rarely. An acute dysenteric disease can be produced in young cats by feeding or injection into the rectum of faeces containing cysts or of material from liver-abscesses.

Diagnosis. The microscopic appearance of the stools differs from that of bacillary dysentery in the comparative scarcity of leucocytes (see p. 95). A drop

of a very fresh stool should be examined on a warm stage for amoeboid movements of the parasite and for ingested red cells; and to another drop a little neutral red should be added, to show up the nucleus. For the identification of cysts a weak iodine solution mixed with some emulsified faecal matter brings out the nuclei well and shows up granules of glycogen in the cytoplasm. The diagnosis is complicated by the frequent presence in human faeces of non-pathogenic amoebae, of which *Entamoeba coli* is the most common. Considerable practice is needed to distinguish between this organism and *Ent. histolytica*. It is generally larger and contains no red-blood cells, but is often full of bacteria and other objects. The cysts, which are also on the large side, have a rather more definite wall, contain anything from two to eight nuclei and seldom show any chromatoid bodies (Fig. 25).

The Pathogenic Fungi

(*Ringworm; favus; thrush; various mycoses.*)

Infection by moulds and yeasts is mainly confined to the lifeless surface-structures, such as hair, nails, and the horny epidermis; though deeper invasions are by no means rare.

The moulds or *hyphomycetes* consist of long, branching, septate threads (*hyphae*) or sometimes chains of cubical or ovoid elements. An aggregate of hyphae is called a *mycelium*. Reproduction is mostly by asexual spores of various types, called conidia, oidia, ascospores, &c., which generally develop at the ends of hyphae, often in special structures called fructifications. Sexual reproduction also occurs, mostly under unfavourable conditions of nutrition.

The yeasts or *saccharomycetes* are round or oval budding cells, which do not form hyphae. Spores are

formed, but are not easily distinguishable from the vegetative cells.

Between the moulds and yeasts there exist intermediate forms with some of the features of both (Fig. 26. C).

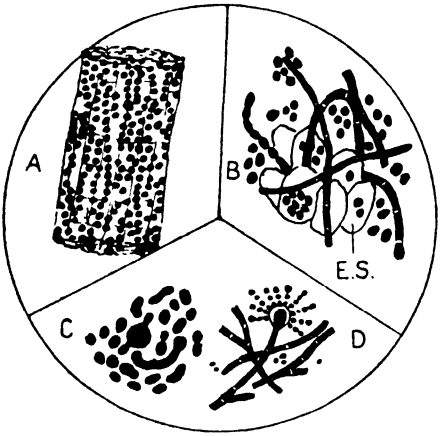

FIG. 26. Some pathogenic fungi

A. *Microsporum audouini* on a hair in Ringworm. B. *Oidium albicans* in Thrush. E.S. = epithelial scales from mouth. C. Yeast-like fungus from blastomycosis. D. *Aspergillus*.

Fungal infections. The living tissues, although not as a rule primarily invaded, are greatly irritated by the products of the parasitic growth and by the secondary invasion of pyogenic cocci; so that suppuration is a common feature. Some fungi, however, definitely penetrate the skin or mucous membranes and give rise to granulomatous lesions (mycetoma). General infection of the blood and internal organs seldom, if ever, occurs.

TABLE XXII

Fungus-infections

Disease	*Commonest Causes*	*Structures infected*	*Microscopic appearances*
Ringworm (Tinea, pityriasis, &c.)	*Microsporum (audouini, &c.)*	Horny layer of epidermis and hairs, chiefly scalp	Fine septate mycelium inside hairs and scales. Spores in rows and mosaic plaques on hair-surface
Do.	*Trichophyton (tonsurans, &c.)*	Hairs of scalp, beard, and other parts. Also nails	Mycelium of chained cubical elements and threads in and on hairs. Often pigmented.
Favus	*Achorion (schönleinii, &c.)*	Yellow disks in epidermis round a hair. All parts of body; also nails	Vertical hyphae and spores in epidermis. Sinuous branching mycelium and chains in hairs
Epidermophytosis (Dhobie itch, &c.)	*Epidermophyton (inguinale, &c.)*	Inflamed patches in inguinal, axillary and interdigital folds. Hairs not affected	Long, wavy, branched and segmented hyphae and spindle-shaped cells in stratum corneum
Thrush and tonsillomycosis	*Oidium or Monilia (albicans)*	White patches on tongue, mouth, and throat. Some inflammation	Large hyphae and yeast-like budding cells in epidermis
Fungal granuloma (Sporotrichosis and blastomycosis)	*Sporotrichon (beurmanni, &c.)*	Inflammatory thickening of skin with suppuration	Oval spores and yeast-like cells in tissues and pus
Do.	*Cryptococcus*	Do.	Budding yeast-like cells, short hyphae and large capsulated spheroids in tissues and pus
Aspergillosis (Pneumomycosis and bronchomycosis	*Aspergillus (fumigatus, &c.)*	Pustules in external ear. Inflammatory and necrotic foci in lungs	Branched hyphae and spore-bearing fructifications in pus or sputum

Transmission is either by direct contact or indirectly through infected clothes or dust containing hairs or epidermal scales.

Diagnosis is made by direct microscopical examination of hairs, epidermal scales, &c., after heating them for a few seconds in a dilute alkaline solution (e.g. 10 per cent. KOH). The form and distribution of the mycelium and spores is sufficiently characteristic in many cases, but if it is desired to ascertain the species accurately, the fungus must be cultivated on a suitable medium such as maltose-agar, and its morphological and cultural characters carefully observed. The fermentation of carbohydrates, &c., is a valuable means of differentiation.

An abbreviated account of the commoner fungal infections is given in Table XXII. It is important to realize that the cause given for each disease is merely the commonest cause. A single fungus may cause more than one type of infection, and a single 'disease' may be caused by several different fungi.

Immunity-response. In superficial infections there seems to be so little absorption of antigens that little or no antibody-production occurs. But, when considerable tissue invasion takes place, precipitating, agglutinating, and complement-fixing antibodies may be formed.

Bacteria of Minor Medical Importance

Fusiformis

(Gangrenous and necrotic lesions; Vincent's angina)

Under this generic title we include a rather miscellaneous group of organisms, all more or less resembling the type-species *Fusiformis fusiformis.* This is a Gram-negative, non-sporing, anaerobic, non-motile longish rod, pointed at both ends, found chiefly in necrotic inflammatory swellings of the throat and mouth (Vincent's angina)

usually in association with a spirochaete, *Trep. vincenti* (Fig. 27). It grows *anaerobically* on the surface of serum or serum agar in small colonies, like a streptococcus, and in serum-broth it forms whitish flocculi with little or no

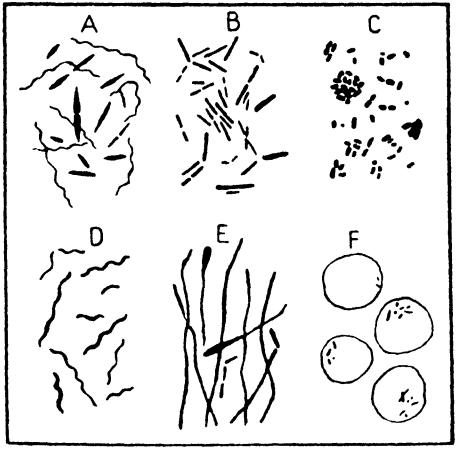

FIG. 27. Bacteria of minor medical importance.

A. *Fusiformis* with spirochaetes. B. *Lactobacillus* (*acidophilus*). C. *Chromobacterium* (*prodigiosum*). D. *Spirillum rubrum*. E. *Leptothrix*. F. *Bartonella* in erythrocytes.

turbidity. It is almost completely non-pathogenic to animals, and its primary role in the human disease is open to question.

Bacterium alkaligenes

(Syn. Bac. faecalis alkaligenes.)

This is a species or perhaps a mixed group of intestinal commensals which occasionally give rise to a septicaemic infection with mild typhoidal symptoms. In general characters they resemble the genus *Bacterium*, but they ferment *no carbohydrates*, and quickly produce an alkaline reaction in milk.

Chromobacterium

(*prodigiosum*, *violaceum*, &c.)

Numerous saprophytic species of small Gram-negative, motile, non-sporing, unencapsulated aerobic rods, which produce intense red, yellow, or violet pigment in massed growth, are to be found in water, soil, and occasionally in the upper respiratory passages and intestines of man and animals.

Chr. prodigiosum is notorious for having been responsible in early times for the miraculous appearance of blood-stains on bread and other foods. These organisms are easy to cultivate on ordinary media; they liquefy gelatin rapidly and usually alkalize, coagulate, and peptonize litmus milk.

Lactobacillus

(*acidophilus*, *bulgaricus*, &c.)

This genus contains a number of species of fairly long and thin Gram-positive, non-motile, non-sporing, unencapsulated aerobic rods (some species are anaerobic at first), which are found in sour milk, in the intestines of animals and man, and in soil. They have the peculiar power of growth at a pH of 3 to 4. They have generally no pathogenic action, but certain species, e.g. *Lact. odontolyticus*, are found in carious teeth in such numbers as to suggest that they are at least partly responsible for the decay. The ready production of lactic and other acids (without gas) from carbohydrates accounts both for the coagulation of milk and for the decalcification of teeth. The great Metchnikoff, who is said to have been morbidly afraid of death, believing that human life might be prolonged if putrefaction in the large intestine could be prevented, advocated a diet of sour milk, with the object of substituting a flora of lactobacilli for the putrefactive anaerobes and other supposedly harmful inhabitants of the colon. *Lactobacillus bulgaricus* has most commonly been used, sour milk being a staple diet in Bulgaria; but there is little evidence that the treatment achieves either its immediate or its ultimate object. An organism of this

genus, *Lact. bifidus*, is found in large numbers in the faeces of breast-fed babies, and another, *Lact. acidophilus* (Döderlein's bacillus), is common in the same place and in the healthy female vagina.

Spirillum

These are spiral, motile, and usually Gram-positive rods, with a flagellum or tuft of flagella at each end, which distinguishes them from spirochaetes. Apart from *S. minus* (p. 187) of rat-bite fever there are no pathogenic species. Of the saprophytic species the best-known is *S. rubrum*, a water-organism which produces a pink pigment on solid media.

Leptothrix

A genus of long, thick, unbranched, thread-like organisms, classed with *Actinomyces* and some others among the higher bacteria, which approximate to the moulds. It includes the interesting iron-bacteria, which give a rust-colour to stagnant water in ferrous soils, and the normal inhabitant of the mouth and tonsillar crypts, *Leptothrix buccalis* (p. 266). This organism is Gram-positive and grows slowly under aerobic conditions on glucose agar or serum-glucose agar, forming tiny colonies in 3 to 4 days. Organisms apparently belonging to this group have been isolated from the cerebrospinal fluid in a few cases of meningitis.

Bartonella

(*Oroya fever*)

In 1915 Barton observed very small irregular rods *inside the erythrocytes* in Peruvian 'oroya fever' and verruga peruviana. The organism has been cultivated in blood media and the disease has been transmitted to monkeys with pure cultures. It is aerobic, motile, and Gram-negative. An organism of similar form and habit causes anaemia in dogs; and another species causes a latent infection in rats, which generally becomes manifest if the spleen is removed.

CHAPTER XIV

ULTRAMICROSCOPIC AGENTS: VIRUSES: BACTERIOPHAGE AND BORDER-LINE ORGANISMS

In many infectious diseases of animals and plants, in which exhaustive research has failed to discover a visible bacterial cause, it has been proved that the infection can be transferred from one individual to another by means of the tissue-juices or exudates freed from bacteria by filtration through porcelain or collodion filters.

These filtrates clearly contain *infective agents below the size-limit of microscopic resolution* (see below), to which the names 'viruses', 'filterable viruses', or 'ultramicrobes' are variously attached.

The study of these entities has been difficult and slow, since they have all until recently been entirely inaccessible to our senses; but the following characters have been established for viruses as a class, though it is admitted that the class may prove to comprise entities of different kinds: (1) they are in general too small to be recognizable by simple microscopy; (2) they pass through filters that retain all visible bacteria; (3) they are solid particles; (4) they propagate only in the living cells of higher organisms; (5) their presence in cells is often shown by histological changes (inclusion bodies); (6) they possess antigens; and (7) they show adaptive variability.

Size, visibility, and resolution. With ordinary transmitted light the strongest microscopic lenses cannot *resolve* the images of objects less than 0·25μ in diameter. Particles as small as 0·075μ may be visible as dots of

indeterminable shape, and with dark-ground illumination (p. 18) the limit is much lower.

Resolution, i.e. the production of a formed image, is limited by the value of half the wave-length of the shortest visible rays ($0{\cdot}4\mu$); but by using shorter, ultra-violet rays, and the camera in place of the eye, the form of particles as small as $0{\cdot}075\mu$ can be determined.

By these methods it has been proved that many infective filtrates contain innumerable minute spherical bodies, which are accepted as the virus itself. Some of the larger ones can be made visible in dry films by staining (e.g. Giemsa), which increases their apparent size.

Filtrability. This implies the ability to pass through porcelain filter-candles or collodion membranes which retain all ordinary bacteria.

Now the passage of minute particles through very small pores is not governed solely by mechanical principles. Owing to electrical attraction or adsorption the tortuous channels of the porcelain retain particles considerably smaller than their own diameter, so long as the charges on filter-substance and particle are opposite. But if we change the charge on the particles by adjusting the reaction of the fluid, or that of the filter by constructing it of different material, the particles will pass through.

Like bacteria, viruses move towards the anode in a neutral fluid subjected to a strong electrical field (cataphoresis). In other words they bear a negative charge.

Since the ease and certainty of filtration are also affected by other variable factors such as the quality of the filter-candles, the presence of particles other than virus, the viscosity of the fluid, temperature, and filtration-pressure, filtrability cannot be considered an infallible criterion for the presence of a virus. Furthermore, certain very thin organisms of considerable

length, such as spirochaetes, and also a number of border-line organisms (see below) will pass through filters that retain all other bacteria.

Ultra-filtration is the name given to filtration through

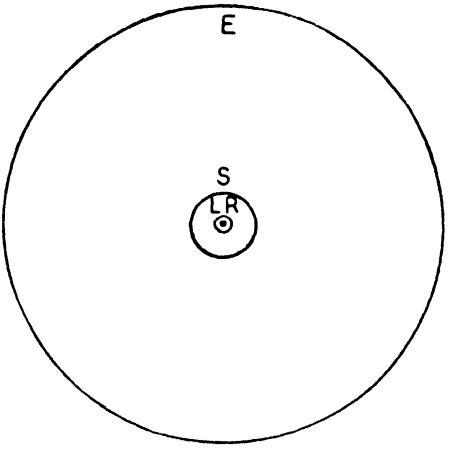

FIG. 28. Diagram of relative microscopic and inframicroscopic dimensions. Virus, staphylococcus, erythrocyte.

E = Human erythrocyte; 7,000 mμ. S = Staphylococcus; 1,000 mμ. L.R. = Limit of resolution; 250 mμ. Central dot = a virus; 100 mμ.

membranes made with graded concentrations of collodion in such a way that their average pore-size can be regulated with considerable precision. This can be further checked by measuring the rate of flow of water at constant pressure, or by testing for the passage of mineral particles of known magnitude. The approximate measurements of viruses made in this way agree fairly well with the microscopic estimates (Table XXIII and Fig. 28).

Centrifugalization. The ordinary, small centrifugal machine is not fast enough to throw down viruses, but the recently developed super-centrifuges, running in a partial or complete vacuum at speeds of 150,000 r.p.m., will separate out viruses and give an estimate of their size and its constancy according to the rate and completeness of sedimentation at given speeds. The fact that virus has been deposited is proved by the infectivity of the deposit to animals and the non-infectivity of the supernatant fluid.

These experiments prove viruses to be particulate, but it is important to remember that proteins under the same stresses behave as particles.

The estimates of size obtained by filtration, centrifugalization, and photomicrographic measurement are in good agreement and give the figures shown in Table XXIII and Fig. 28.

TABLE XXIII

Estimated approximate diameters of certain particles

1 mm. = 1,000μ = 1,000,000 mμ.

Human erythrocyte	7,000 mμ.
Staphylococcus	1,000 mμ.
Border-line bacteria Very large virus (psittacosis)	300 mμ.
Large viruses (e.g. vaccine; herpes)	100–150 mμ.
Medium viruses	30–80 mμ.
Small viruses (e.g. poliomyelitis; foot and mouth)	8–20 mμ.
Serum proteins	6 mμ.

Border-line organisms. Certain minute organisms resemble bacteria in having a just visible coccoidal or cocco-bacillary form, but would be classed as viruses if filtrability were the sole criterion. The best known of these are the microbe of bovine pleuro-pneumonia and *Bacterium pneumosintes*, a harmless inhabitant of the human respiratory tract. In addition

we have the *Rickettsiae* (after Ricketts, their discoverer), a very interesting group of visible but not 'filtrable' minute rods or coccoids, 0·5μ or more in length, which are seen in Giemsa-stained preparations of the cells in typhus fever, Rocky Mountain spotted fever, trench fever, and probably trachoma. There can be little doubt that they are the real cause of the typhus group of fevers, since they can be found in myriads in the intestine of the blood-sucking lice that transmit those diseases and in the tissues and exudates of guinea-pigs after experimental infection with the blood of human patients. But Koch's second postulate (p. 46) remains unsatisfied, since, like the viruses, the *Rickettsiae* have hitherto resisted all attempts at cultivation except in live tissue-cultures, where they live and multiply in the cytoplasm or even in the nuclei of the cells. Filtrable ultra-microbes of the same order of size as the larger viruses, but non-pathogenic and capable of growth in tiny colonies on ordinary media, have recently been isolated from sewage. These can hardly be called viruses, since the latter are by definition infective agents.

Commensal viruses. There is some evidence that the cells of certain animal-organs are infested with 'viruses' which cause no obvious lesions. The cells show 'inclusion bodies' and the condition seems to be transmissible by the injection of tissue suspensions.

Resistance to chemical and physical agents. The effects on viruses of heat, cold, radiation, desiccation, and disinfectants lie, so far as is known, within the same range as their effects on the vegetative cells and spores of bacteria. Though most viruses are just as sensitive as vegetative bacteria to heat and radiation (except bacteriophages which survive temperatures that kill the host-bacteria), many viruses rather unexpectedly show a resistance to chemicals approaching

that of bacterial spores. Like bacteria, however, they show great individual differences of resistance to different agents. Some are inactivated by 0·1 per cent. of formalin while others resist twice that concentration for long periods. Phenol at 0·5 per cent. or even 1 per cent. cannot always be relied on to kill a virus, but we must remember that the latter generally has the protection of proteins in the filtrate. A higher resistance to 50 per cent. glycerine than that of most vegetative bacteria is a character of the vaccinia and some other viruses, and this is the basis of the preparation of vaccine-lymph (which, however, may contain living *Staphylococci*, &c.). Desiccation is a very useful method of preserving viruses, but it is also applicable to bacteria.

Growth and multiplication of viruses do not take place in ordinary culture-media. But in 'tissue-cultures' of suitable living cells infected with virus a progressive increase of the infectivity of the culture gives evidence of a multiplication or regeneration of the active agent. A very useful recent 'medium' for propagation is the chorio-allantoic membrane of developing hen's eggs. Most, but not all, viruses multiply enormously and cause progressive lesions in the membrane and embryo. This method is now greatly used in place of animal-injections for the maintenance and mass-production of viruses. It has recently been shown that certain animal-viruses (vaccinia, foot and mouth) will live and propagate 'symbiotically' in yeast-cultures. This provides interesting possibilities of dissemination.

Cell-inclusions and elementary bodies. Viruses are essentially cell-parasites. In many of the virus-diseases the cells of the diseased organs when examined histologically show abnormal bodies included in the cytoplasm and sometimes in the nucleus (Fig. 29).

These inclusions have been given different names in different diseases: Negri bodies in rabies; Guarnieri bodies in small-pox, and the like. They are irregular in size, shape, and number. Though usually quite small, they may sometimes equal the nucleus in size,

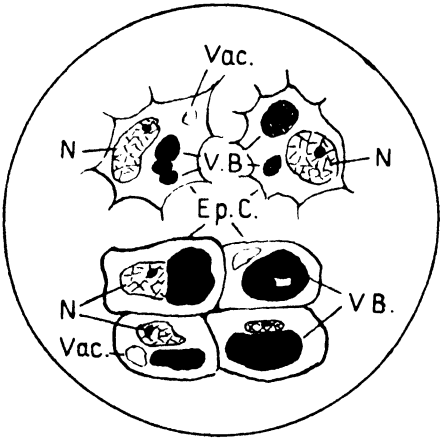

FIG. 29. Cell-inclusions in virus diseases.

Ep. C. = Epidermal cells of chick artificially infected with vaccinia virus. V.B. = Virus bodies (cell inclusions). N. = Nucleus. Vac. = Vacuole. (After Ludford.)

from which, however, they are generally distinguishable by a greater affinity for eosin. Until lately it was a matter for speculation whether they are peculiar degeneration-products or in some obscure way represent the virus; but experiments in which they have been crushed, stained, and examined with very high magnification, or photographed by ultra-violet light, have shown that they consist of innumerable spheroidal particles of more or less uniform size. It has already been mentioned that similar minute spheroids, the

'elementary corpuscles', may be seen in Giemsa-stained preparations from tissues and exudates in several virus-diseases, and there is little doubt that these particles are actually the virus. They can, for example, be specifically agglutinated by a suitable antiserum. Inclusions have not been demonstrated in all virus diseases.

Antigenic properties. The injection of virus-containing filtrates into animals gives rise to antibodies which, as we have just seen, will *agglutinate* the 'elementary bodies' after the latter have been freed from cell debris and extractives by repeated washings and fractional centrifugalization. The *complement-fixation*-reaction is also given by these serums with the corresponding virus. As we shall see later, a beginning has already been made in the laboratory-diagnosis by these methods of large-virus diseases such as small-pox and chicken-pox. The *precipitin reaction* is also given by filtrates freed from elementary bodies, and in the case of variola and vaccinia there is good evidence that the active precipitinogen is a polysaccharide-hapten like those of bacteria. Finally, there is convincing evidence of antigenic differences among different races of a given virus, e.g. the foot-and-mouth and influenza viruses, in that immunity against one race is ineffective against another.

The general nature of antivirus immunity is discussed on p. 246.

Variation and adaptation. The close relation but lack of identity of two or more 'races' of virus to each other is analogous to the variation of other living creatures. To take an example: the virus of small-pox is capable of infecting cattle, causing a similar but milder pustular disease (cow-pox), in the course of which it is so changed as to be incapable of causing *small-pox* in human beings; it now gives rise to the

much less severe, localized *vaccinia*. But its chief antigenic properties remain intact, so that an artificially induced attack of *vaccinia* (vaccination) produces in human beings a solid immunity against the original virus of small-pox. Further, it seems that in the disease *alastrim*, which is indistinguishable from a mild small-pox, we have a variant race of the same species of virus, which has permanently lost some of its virulence.

Animal and vegetable virus-diseases show many other similar phenomena, which seem most easily interpretable as examples of variation and adaptation of a living organism.

Virus-diseases: The main virus-diseases of man and domestic animals may be classified as follows:

Dermotropic (affecting mainly the skin). Variola (small-pox); vaccinia (cow-pox); varicella and herpes zoster; lymphogranuloma; trachoma; foot and mouth disease.

Neurotropic (affecting mainly the nervous system). Rabies; poliomyelitis (infantile paralysis); encephalitis lethargica.

Catarrhal and general. Measles; german measles; mumps; influenza; yellow fever; dengue; psittacosis; distemper (dogs, &c.).

Neoplastic. Warts; molluscum cantagiosum; sarcoma of fowls; papilloma of rabbits.

The theory that a virus is concerned in human *cancer* is not improbable but remains unproven. The transmissible agents of the various Rous-sarcomata of chickens have the properties of viruses, each of which produces a histologically characteristic type of neoplasm. Again, the generally benign papillomata of rabbits caused by Shope's virus not uncommonly became malignant. Much of the experimental data on cancer, however, suggests a biochemical rather than a microbial cause.

Transmission. Viruses are transmitted, like bacteria, either directly by droplets from the throat, as in poliomyelitis, influenza, and colds; or by fluids, crusts, or scales from lesions and rashes, as in the eruptive diseases, variola, vaccinia, varicella, and herpes; or indirectly by biting insects (e.g. yellow fever) or biting dogs (rabies).

The lesions and symptoms of virus diseases do not differ fundamentally from those of bacterial infections. But the intracellular habit of viruses gives the lesions in most cases the special character already mentioned (inclusion-bodies). A common early lesion is a perivascular infiltration with small mononuclear leucocytes. Suppuration is not a common characteristic of virus-infections, though viruses very often prepare the soil for secondary pyogenic infection (e.g. streptococci in measles and influenza).

Some typical virus-diseases

Variola (small-pox) and Vaccinia

Although the march of civilization and hygiene has removed small-pox from the list of major European pestilences, it still takes a great toll of life in the East, especially India where the annual incidence is some 250,000 cases with a 20 per cent. fatality. The main *symptoms* are fever, serious prostration, and a widespread skin-eruption of vesicles soon developing into pustules or pocks.

The virus is widely disseminated in the body and blood, but is specially concentrated in the epidermal lesions, where the cells show numerous inclusion-bodies (Guarnieri corpuscles).

Infection of animals. Apes and monkeys can be given small-pox by inoculation of infected human pus or blood, but in other mammals, such as calves or

rabbits, a most interesting and important phenomenon occurs; the inoculation gives rise only to local lesions, and if the virus is passed through several animals in succession it is found to have lost its power of producing anything more than similar local lesions in man, which are called *vaccinia* (i.e. cow-pox). On recovery from these lesions, however, the subject is immune not only to the altered vaccinia virus, but also to the parent virus of variola. It was on this basis that Jenner built the present system of vaccination by scarification of the skin with glycerinated calf-lymph containing the altered, living virus. Most mammals suffer naturally from 'pox' due to viruses closely related to that of variola. From most or all of these a variant identical with the vaccinia virus can be produced by calf-passage.

Transmission of the virus from man to man is by droplets of mucus or by pus and crusts from skin-pocks.

The virus is one of the larger ones, about 150 mμ. It will generally, though not always, pass through bacterial filters, and it can be seen in stained preparations of infected cells or pus in the form of minute spheres (elementary bodies or Paschen granules). It can be propagated both in living tissue cultures and on the chorio-allantoic membrane of developing hen's eggs.

Immunity. An attack of small-pox confers a solid, almost life-long immunity. Agglutinating, precipitating, and complement-fixing bodies are present in the serum of convalescents and inoculated animals, and a diagnostic reaction between a suspected serum and a suspension of known pustular matter is practicable. Small-pox can be distinguished by this means from chicken-pox.

Vaccination. Some time in the eighteenth century Lady Mary Montague studied in Constantinople and

subsequently introduced into England a method, long practised in the East, of preventing small-pox by direct case-to-case inoculation. The discharge or scab from a pustule of a mild case was scratched into the skin of the person to be protected; a mild localized eruption followed, and on recovery the subject was safe from further attack. Accidents, however, were not uncommon, and led to the safer Jennerian method already described.

Jennerian vaccination is universally accepted as an effective prophylactic, though statistically indisputable figures are scanty. The reduction of *incidence* is illustrated by U.S.A. statistics in 1919–28: in states where vaccination was rare 115 per 10,000 persons contracted small-pox, whereas in compulsorily vaccinated states the incidence was only 7 per 10,000.

A lowering of fatality is seen in the figures from London fever hospitals in 1901–4, where the percentage fatality of cases of small-pox was less than 10 in vaccinated persons, but 31 in the unvaccinated. Recent attempts at immunization with vaccine killed with formalin, &c., have not been successful.

Alastrim or Variola minor is a disease like small-pox, but very much milder. Well known in the East, it has lately been prevalent in Europe. It is almost certainly due to a different virus, and the two infections give no immunity against each other. *Chicken-pox* and *Herpes zoster* are two manifestations of infection with a relatively harmless virus of the same general type as Variola.

Poliomyelitis (infantile paralysis)

Because of its legacy of withered limbs this disease is dreaded out of all proportion to its frequency or fatality. Endemic in all parts of the world, it breaks out here and there in considerable epidemics. Children are most susceptible, but no age is completely immune.

The *symptoms* are fever, headache, and stiffness of neck and back, often followed by paralysis of various groups of muscles and sometimes by death. Abortive infections with minimal symptoms are believed to be common. The characteristic *lesion* is a degeneration of the anterior-horn cells of the spinal cord and medulla, due to the growth of the virus and resulting in a subacute inflammation with 'cuffing' of the small blood-vessels. The virus is present in the nerve-cells and may also be demonstrated by animal experiment in the blood, in filtrates of nasal washings, and in the faeces. The only animals that can be infected are our nearest relations, the apes and monkeys.

The presence of the virus in the nasopharynx, and the possibility of infecting monkeys by nasal insufflation point to droplets as the normal mode of *transmission*. From the infected nasopharynx the virus most probably penetrates the olfactory nerves, passes up them direct into the brain, and thence to the spinal cord. Infection through the intestinal tract is another possibility. The virus has been found from time to time in the nasopharynx of healthy persons who have recovered from or been in contact with infection. Such chronic *carriers* are an obvious danger to all.

The Virus, if a living organism, is almost incredibly small; about 10 mμ or one hundred-thousandth of a millimetre. It does not seem to produce inclusion-bodies, nor visible elementary bodies, but it can be propagated, like other viruses, in living tissue cultures.

Immunity. The natural immunity of the human race seems to be strong, judging from the sporadic nature and limited spread of the infection. Immunity following recovery is solid and durable. The serum of convalescents and monkeys after recovery often contains antibodies which neutralize the virus when

simultaneously injected into monkeys; but serum from subjects not known to have had the disease or contact with it may also contain specific antibodies, probably due to widespread subinfection and undiagnosed infections. It is not at all clear that antibodies play a primary role in the immunity, or how they could prevent infection along nerve-fibres. A special type of 'cellular immunity' has been suggested to account for the phenomena (p. 247). Much therapeutic use has been made of convalescent serum, injected when possible in the preparalytic stage of the infection, but its efficacy has been called in question by recent American statistics. Continued attempts are being made to produce a 'killed' or attenuated virus for *active immunization* by treatment of a suspension of infected monkey-cord with 1 per cent. sodium ricinoleate of 0·1 per cent. formol, but as yet neither immunity nor safety has been achieved.

Rabies

Rabies or Hydrophobia is a natural infection of dogs, cats, and certain wild carnivora with a neurotropic virus which multiplies in the nerve-cells of the brain, producing cytoplasmic inclusions (Negri corpuscles), and causing madness and death. It is also present in the blood and saliva, through which it is transmitted to man by bites. Filtration through porcelain etc. is sometimes, but not always, successful. The size of the virus has not been determined, and it has only been cultivated a few times in tissue-cultures of ganglion-cells.

The Pasteurian treatment for bites by rabid dogs is a classic of immunological pioneer work. By a daring experiment on a bitten child he showed that during the 6–8 weeks' incubation period it is possible to induce *immunity against rabies* by a course of injections of

attenuated virus. A graded series of viruses, each weaker than the last, is prepared by drying the spinal cords of artificially infected rabbits for increasing lengths of time. Beginning with the weakest, the course finishes with an only slightly attenuated virus, and by this time the patient's resistance is raised sufficiently high to deal with the flood of virus released at the end of the incubation-period. This method is still in use, though attenuation by chemicals or heat is practised in some places. The use of living vaccine is justified by the danger of the situation; unless treated in time, about a third of those bitten contract rabies and die.

Influenza

Epidemic influenza, though generally a benign disease, is one of our most insistent plagues, and in association with virulent cocci or *H. influenzae* it may take a very malignant form. During 1918–19 in India alone its millions of victims are believed to have greatly outnumbered those of the whole world-war. Clinically, it is difficult to distinguish from febrile catarrh and other fevers, owing to the indefinite character of the symptoms, which are: sudden malaise and fever, within 48 hours of infection, with pains in the muscles of the back and eyes, followed some days later by catarrh which may progress to bronchitis or pneumonia. An illness starting with a cold in the head is seldom, if ever, true influenza.

Transmission is by droplets, and the virus can be demonstrated in filtered nasopharyngeal washings by nasal instillation in man, ferrets, or mice. In the mouse, after a few passages it causes characteristic lung-lesions in which the virus is often the sole micro-organism. It seems that ferrets can take the infection from man and man from the ferret. Seasonal out-

breaks are common in the late winter, and in the intervals the virus is kept alive by sporadic cases and perhaps by carriers.

The Virus. Our knowledge of the virus is of very recent and mainly English growth. Ultrafiltration gives it a diameter of 80–120 mμ. It can readily be propagated by the egg-method, but in the process it loses its virulence for man, though apparently not its immunizing power. No animals other than the ferret are naturally infected by the human virus; but several animals have an 'influenza' caused probably by other viruses.

Immunity. On recovery from the induced disease ferrets are immune for several months, and their blood contains neutralizing antibody. The serum of human convalescents (but not convalescents from other infections) has a similar neutralizing power.

Active immunization of ferrets and mice with mouse-lung virus inactivated with 0·02 per cent. formalin gives effective resistance, and experiments on man are now in progress. A difficulty, recently met with, lies in the probable existence of several serological types of the virus.

Colds. There is good evidence that the common cold, or one type of it, is caused by a virus distinct from that of influenza. Bacteria, however, also play a large part in colds, whose causation is by no means fully understood.

Yellow fever

This is a very dangerous jaundice-producing infection endemic in West Africa, South America, and the West Indies, which causes severe necrotic inflammation of the intestinal tract and liver. Infection is transmitted by the mosquito, *Aedes aegypti*, as can be proved by causing it first to bite an infected patient and then a Macacus monkey. An interval of about 12 days is

needed for the mosquito to become infective. The patient's blood also is infective, even after porcelain-filtration, which proves the cause to be a virus. Although no animal other than certain monkeys will take 'yellow fever', the virus can be propagated in the central nervous system of living mice, where it causes an encephalitis. The original 'viscerotropic virus' has thus become 'neurotropic' and has lost much of its virulence for man. The virus is relatively small, 17–28 mμ, and not microscopically demonstrable. Prophylactic immunization with virus attenuated or devitalized in various ways is now on trial.

Jungle yellow fever is an almost identical fever recently shown to be prevalent in wild monkeys and man in jungle districts. The insect vector is not *Aedes aegypti* but another insect or insects, and the relation of the virus to that of the urban type has not yet been worked out.

Measles

The proof that this is a virus infection rests on the transmission of the disease to monkeys and man with blood or nasopharyngeal washings of measles patients, freed from bacteria by filtration. The virus itself has not yet been adequately studied.

Immunity. An attack of the disease generally confers solid and prolonged immunity. The serum of convalescents contains antibodies which protect human beings from measles if injected soon after exposure to infection. If given later in the incubation-period it greatly reduces the severity of the subsequent attack (attenuation), without interfering with the development of immunity. The serum of normal adults has a similar though probably rather weaker protective action, and so has an extract of normal placenta. It is assumed that the action of both is due to antibodies acquired in early life.

Transmissible lysis of bacteria. Bacteriophage

A remarkable class of viruses, which kill and dissolve bacteria, has been shown to exist in 'sterile' filtrates of sewage and animal excreta. A drop of such a filtrate

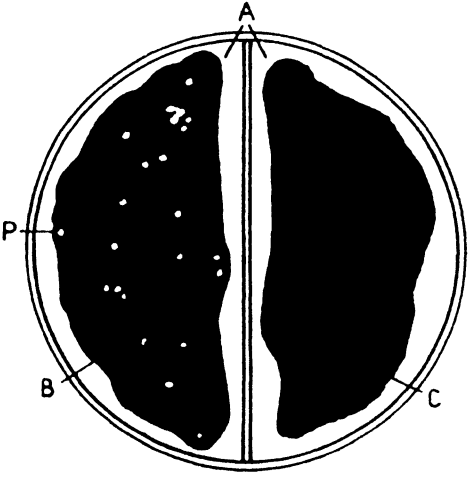

FIG. 30. Bacteriophage plaques.
A. Agar-medium in a Petri-dish.
B. Film of bacterial growth showing plaques (P).
C. Control film of bacteria without bacteriophage.

added to a young, growing broth-culture of a susceptible organism, causes, after a short period of incubation, a partial or complete clearing of the culture. Similarly, if a drop is mixed with a suspension of the bacterium and the mixture spread on an agar surface, the resulting film of growth shows numerous transparent areas or 'plaques' of lysis. During the process the lytic agent is regenerated; and it can thus be

propagated indefinitely by adding a trace of the dissolved culture or culture-filtrate to a fresh tube of living bacteria and repeating the process as often as required.

Lysis only occurs in actively growing cultures; in the stationary phase of growth there is little lysis; in dead cultures none. Different strains of the agent have different ranges of bacterial species or varieties on which they act, but a strain can sometimes be 'trained' to act on a variety of bacterium on which it previously had no effect. Cultures that have undergone lysis are seldom completely sterilized. Some few cells survive, and from them *secondary cultures* can be raised which are *resistant* to the lytic agent. These cultures are often found to be *lysinogenic*, i.e. to give rise on filtration to the agent, though they themselves are immune.

In some species of bacteria a large proportion of stock strains have been found to harbour a bacteriophage, to which the strain itself is resistant, but which will lyse other strains or species.

The number of different bacteriophages is enormous; e.g. some fifteen different phages specific for *Vib. cholerae* have been isolated. Some phages are so narrowly specific as to act only on variants or phases in which a given antigen is present, e.g. a phage is known that acts only on the Vi antigen of *Bact. typhosum* (p. 86).

Like other viruses, bacteriophages are antigenic, producing specific inhibitory antibodies distinct from those against the antigens of the bacteria on which the phage is propagated.

The *size* of bacteriophages, as proved by ultrafiltration, varies from about 10 mμ to about 75 mμ.

Bacteriophage-therapy. The bacteriophage was at first hailed as an antibacterial therapeutic agent of

revolutionary importance. Destructive to bacteria and harmless to man, what could be a more perfect way of ridding him from cholera, plague, or dysentery? But the brilliant results reported by the pioneer workers in these diseases have not been generally confirmed, and all attempts to prove the sterilizing action of the agent on experimental infections of animals under properly controlled conditions have been failures. Nevertheless, the method of treatment has many active supporters, who attribute the failure of others to inadequate technique and the use of insufficiently active strains of bacteriophage. The situation therefore is still full of interest.

The nature of Viruses

Viruses resemble pathogenic bacteria in their infectivity, their power of multiplication, and their adaptive variability. They have also much the same range of resistance to chemical and physical agencies such as heat, radiation, desiccation, and disinfectants. They differ from bacteria mainly in being unable to grow on a lifeless medium, in having, so far as can be ascertained, no independent metabolism and in ranging down to a size-limit hardly exceeding that of known protein molecules. In fact, it has been recently claimed on good experimental evidence that certain tobacco- and tomato-plant viruses are actually proteins of large molecule and peculiar properties, and that one at least of them is crystallizable in the form of rhombic dodecahedra. If this proves true, a new category of infective proteins will emerge and the resemblance between viruses and enzymes will be at least as striking as that between viruses and bacteria. This resemblance to enzymes has hitherto been stressed more in connexion with the viruses of bacteria (bacteriophages) than with those of animals or plants.

At the outset of bacteriophage research Twort, one of the original discoverers, suggested that the agent might be an autocatalytic enzyme, and Bordet exploited this view very thoroughly, while d'Herelle insisted that it was a living ultramicrobe. A third theory pictured the bacteriophages as vital particles, analogous to the genes of animal and plant chromosomes, which are capable of transmission from one cell to another, causing an autolytic crisis with reproduction of similar particles.

The identification of certain viruses with large proteins, if it gains acceptance, will reopen the problem of the meaning of life, for we shall either have to stop calling viruses living things or to admit that a mere protein can live. Such a mental readjustment will be made easier by the reflection that we have never understood the difference between the living and the dead.

CHAPTER XV

THE DEFENCES OF THE BODY: IMMUNITY, HYPERSENSITIVENESS, ANAPHYLAXIS

THE self-protective mechanisms and reactions of the body against bacterial invasion may be considered under the following heads:

(1) *External or surface defences*, which tend to prevent the penetration of microbes into the tissues.

(2) *Primary internal defences*, which we shall consider under the heads of *Natural cellular immunity*, *Phagocytosis*, and *Natural antibodies*. Defensive agents of this class, being part of the normal constitution of the body, are largely non-specific. They are, however, immediately available. Since some bacteria resist their destructive action (virulence is an expression of this resistance), a second line of defence is necessary.

(3) *The secondary, specific defence-reaction*. This consists in the production of large quantities of specific antibodies, which greatly enhance the phagocytic and antibacterial power of the blood against the invading microbe. There is, however, a considerable delay in its operation. Its end-result is *acquired immunity*.

External or surface defences

The horny layer of the skin offers an impenetrable barrier to bacteria, though the hair follicles and sweat-glands afford a channel of penetration for pyogenic cocci and certain other organisms. On mucous membranes the bacteria are caught in the viscid, growth-inhibiting secretion which is continually shifted by ciliary or peristaltic action towards the exterior or down into the stomach, where the microbes are mostly destroyed by the strong acid of the gastric juice. The

throat, eyes, and nose, which are the main portals to the interior, are periodically purged by reflex coughing, crying, and sneezing.

Lysozyme. This is a natural bacteriolytic ferment, present in most animal and vegetable tissues, and especially strong in tears, nasal mucus, and sputum, which seems to play a part in surface defence. Though its most powerful action is on certain non-pathogenic cocci, it appears to kill or inhibit many pathogenic organisms strongly enough to be a useful defensive weapon. Apart from the mechanisms just described the mucous membranes do not offer any considerable barrier to penetration by bacteria.

Primary internal defences

Natural cellular immunity. The introduction of non-pathogenic bacteria into the human or animal body, even in large numbers, has no ill effect, because they possess no constituent or secretion which is toxic to human cells. Being foreign particles, they are rapidly taken up and removed from the circulation by phagocytes.

Since toxicity depends on chance chemical affinities between microbe and animal-cell, it is not surprising that a microbe which is harmless to one species of animal may be virulent to another; for every animal and every microbe has a different specific chemical constitution. Poisons of all kinds show a selective action on different animals. Rabbits, for example, are twenty times more susceptible than cats to cobra venom. Now it has been proved experimentally in this case that the difference of susceptibility resides, at least partially, in the cells themselves, as distinct from the blood. Thus, an excised rabbit's heart, freed from blood and perfused with Ringer's fluid, to which various doses of cobra venom are added, ceases to

beat at a dose four times smaller than is required to stop a cat's heart under the same conditions. Similarly, the various tissues of the body show different degrees of susceptibility or resistance to particular bacterial toxins (e.g. nerve-cells and tetanus toxin, p. 168).

Phagocytosis and opsonic action. The primary reaction of the tissues to bacteria and other irritants is inflammation. This involves the attraction and retention of leucocytes in the affected part, together with plasma rich in substances (opsonins), which prepare the bacteria for ingestion and subsequent digestion by the phagocytic cells. We shall see in the next section that certain other properties of the blood assist in the destruction of the bacteria, but it is now generally agreed that phagocytosis plays by far the most important part.

Our knowledge of the phagocytic functions of the leucocytes is mainly due to Metchnikoff (1845–1916), and that of opsonic action to Almroth Wright.

In acute pyogenic infections the most active phagocyte is the polymorphonuclear cell or microphage. In the later stages of such infections, and almost from the first in chronic diseases like tuberculosis, large mononuclear cells of endothelial type (macrophages or histiocytes) act both as direct phagocytes to the bacteria and indirectly by ingesting damaged microphages containing living microbes.

The ingestion of foreign particles in general is a physiological function of these cells, comparable to the feeding process of amoebae, but they can only exercise it with the help of certain constituents of the plasma. The function of these substances is called *opsonic action*, and with respect to this particular function the substances are termed *opsonins* (from the Greek 'to prepare food'). If leucocytes are washed free from serum and suspended in salt solution with

pathogenic bacteria, no phagocytosis takes place; but when a little fresh serum is added and the mixture is warmed to blood-temperature, the bacteria are rapidly ingested. It is on the bacteria, not on the cells, that the opsonin acts, and it acts by combining with the antigens of the cell-membrane, where it forms a coating of globulins of reduced electrical potential, over which the protoplasm of the leucocyte can readily flow. Thus, if bacteria are treated with opsonizing serum and then washed well, they will be ingested by washed leucocytes in the absence of free serum; but leucocytes treated in a similar way with serum and then washed will not take up washed bacteria unless more serum is added. In the case of avirulent bacteria and other inert particles phagocytosis may occur to a small extent in the absence of serum, but this exception does not vitiate the general rule.

Intracellular digestion. The bacteria are usually killed and dissolved in the cells without much delay; but some organisms, e.g. *Myc. tuberculosis*, resist digestion strongly, and probably multiply inside the cell, which remains alive and active for a time, and may disseminate the infection by wandering away and dying in a distant part of the tissues.

The ability of the phagocytes to deal effectively with a bacterial invasion depends chiefly on the virulence of the bacteria. If this is of a low order, the phagocytes generally succeed in destroying the invader at the site of infection. Bacteria of high virulence, however, have the property of resisting phagocytosis to a considerable degree, and are therefore able to multiply and spread in the tissues. If this process is not checked in time, some of the bacteria will sooner or later escape through the lymph-channels into the regional lymph-nodes. Here they meet with a second phagocytic barrier in the form of endothelial phago-

cytes (*macrophages*) lining the sinuses. If this barrier is penetrated, the organisms arrive in the blood-stream where, as we shall now see, their fate again depends largely on their virulence.

The injection of bacteria of various kinds into the blood of animals has established the following principles. If the bacteria are non-pathogenic or of very low virulence, they disappear quickly and permanently from the blood, being removed by phagocytes of the reticulo-endothelial system (p. 241) in the spleen, liver, and other organs. With fully virulent microbes (e.g. pneumococci injected into rabbits) the course of events starts in the same way but ends differently. Here a partial or even apparently complete clearance of the blood is followed after an interval by a reappearance of the microbes, which multiply rapidly and set up a fatal septicaemia. But if we raise the resistance of the animal by injections of a specific vaccine (p. 248), it may now be able to deal with the virulent organism as effectually as the normal animal deals with an avirulent one.

In human infections the escape of bacteria into the blood will have one of several different results, according to the virulence of the microbe and the resisting power of the host: (1) No definite ill-effect. (2) a fresh localization in one or other organ, or (3) septicaemia, i.e. a progressive infection of the whole vascular system.

Natural antibodies. As was first recorded by Lister, normal blood-serum has the property of self-sterilization. Non-pathogenic organisms and also certain pathogenic ones are destroyed in large numbers, though on some pathogenic species, such as virulent pyogenic cocci, it has little or no effect. The *bactericidal power* of a serum may be estimated by mixing it in graded dilutions (after heating to destroy its

complement) with a constant volume of a living bacterial suspension and some guinea-pig complement. After a short incubation at 37° C. each tube is plated out on agar, and the highest dilution of the serum that shows a reduction in the number of colonies is taken as its bactericidal titre. Immune bacteriolysins are measured in the same way. In the days before the supreme importance of phagocytosis was recognized, these direct antibacterial actions of the serum were regarded as the chief explanation of natural immunity. It was supposed that if an animal is naturally insusceptible to infection by a given microbe it must be because its serum is bactericidal to the microbe; and conversely that susceptibility implies a lack of bactericidal power. This, however, has proved not to be a general law. For example, rabbit's serum is bactericidal to *Bac. anthracis*, yet the rabbit is highly susceptible to experimental anthrax. Similarly, the bactericidal action of human serum on *Bact. typhosum* is no guarantee against typhoid fever.

In addition to bactericidal action normal serum has a variable *agglutinating* and *opsonizing* effect on bacteria. These differ from the action of specific immune antibodies in being much less intense (exerted only in low dilutions such as 1 in 10) and more difficult to demonstrate as specific.

The nature and origin of natural antibodies. Although our knowledge is rather uncertain we can with some confidence distinguish two factors:

(1) *A non-specific globulin* which is readily adsorbed on foreign particles in general. By reducing their surface potential this tends to *agglutinate* or *precipitate* them and also makes them easier for the *phagocytes* to ingest. This non-specific factor is needed to explain the agglutination by a normal serum of bacteria with which the owner of the serum can never have been in

contact, e.g. English cow and *Vib. cholerae*. It also makes it easier to understand why bacteria in general and also inert particles, such as mastic, are more highly agglutinated by the normal serum of some animals (e.g. horse and cow) than of others (e.g. guinea-pig and man). The former group evidently have a more active non-specific substance than the latter. This substance is probably the same as complement (p. 180). The fact that the complementary power of a serum is more easily destroyed by heat and chemicals than its other powers does not force us to postulate more than one active substance.

(2) *Specific or semi-specific antibodies* against various bacteria, functioning as bactericidins, agglutinins, opsonins, &c. Small quantities of specific antibodies are formed during the individual's life in response to subinfective doses of pathogenic organisms such as he is bound to pick up from time to time. The quantity and variety of such antibodies will depend on the length and type of life he has led. Children reared in squalor grow richer in antibodies than the sheltered progeny of the well-to-do (p. 134). Although in reality this is 'acquired immunity', it is generally treated under the head of natural immunity because it is rarely possible in any given case to prove that the antibodies have actually been acquired. There is, however, ample evidence that individuals in contact with infections such as diphtheria, whooping-cough, and enteric fever may develop antibodies without showing any symptoms.

By a semi-specific antibody we mean one that is formed in response to a stimulus by an antigen different from, but having an accidental chemical resemblance to, the antigen of some pathogenic microbe. Stimuli of this kind may arise in the digestive tract from food or from the antigens of non-pathogenic organisms. We know that certain antigens can give

rise to antibodies which react with certain apparently quite different antigens; for example, the serum of a rabbit injected with tissue-suspensions from various animals, or with *Bact. dys. Shiga*, will haemolyse sheep's erythrocytes. Such a serum is said to contain *heterophile antibody*. In this case the chemical connexion is unknown; but it is clearer in the case of the immunization of animals with synthetic proteins, which has shown that the possession of a common hapten-radicle by two otherwise dissimilar proteins gives them a common immunological character.

When acting as *opsonins* the small concentrations of specific antibodies in 'normal' serum are generally insufficient to bring about phagocytosis experimentally without the aid of the non-specific complementary substance. When, however, their potency is increased by infection or immunization they can be shown to exert opsonic action by themselves, that is, to act in heated immune serum without the addition of any fresh serum. In this capacity they are sometimes termed *bacteriotropins*.

Conditions influencing natural immunity. Heredity is rightly believed to be a powerful factor. Natural cellular immunity (p. 228) is clearly a genetic character of species or race. Although there is no evidence that the possession of any given specific antibody is an inherited character (congenital transference by the mother is another matter, see p. 134), the genetic constitution determining immunity to a given infection undoubtedly varies widely not only in species, but in races, families, and individuals. Unfortunately, we have almost no knowledge of the matter as it affects man, but research on small animals has given important information. For example, different breeds of mice have been shown to possess different degrees of resistance to *Bact. aertrycke*; and stocks with ab-

normally high immunity to that microbe have been reared by breeding from picked individuals. But the immunity is restricted to the test-microbe and may be accompanied by lowered resistance to others. Thus there is no evidence that a general immunity can be produced by selective breeding.

The well-known sensitizing effect of *cold* is experimentally illustrated by the breakdown of the natural resistance of certain birds to *Bac. anthracis* when they are chilled in cold water. On the other hand, the cold-blooded frog, which is naturally insusceptible to anthrax, can be made susceptible by heat. Another generally accepted contributory cause of chronic diseases, such as tuberculosis, is *malnutrition*, including vitamin-deficiency. Although we do not as yet know very much on the latter subject, one fact at least has been proved experimentally: viz. that a lack of vitamin A in the diet lowers the resistance of the ocular and respiratory mucous membranes of animals and man to infections with pyogenic cocci. Conversely, there is little doubt that a full diet, good housing, and adequate rest help to maintain a high level of resistance. Experimental evidence indicates that ultra-violet radiation does not heighten resistance to infection.

Exhaustion may cause a lighting up of a latent infection, though it does not appear to increase susceptibility to a fresh one.

The secondary, specific defence-reaction

The primary defence-mechanisms we have described, being unable to cope with microbes of high virulence, must be strongly reinforced in some way if the invasion is to be defeated. This reinforcement takes the form of a production of powerful antibodies acting specifically on the invading organism. Unlike the

primary mechanisms, which are always ready, the specific response takes time to develop; so that it is generally some 5 days before any increase in antibodies can be demonstrated, and anything from 10 days to 3 weeks or more before it reaches its zenith.

The secondary defence mechanisms differ from the primary in the greatly increased power due to the specific antibodies. In the blood and tissue-fluids the surfaces of the microbes soon become coated with the specifically combining antibodies, which agglutinate them, precipitate their toxins, and enable the phagocytes to ingest them faster than they can multiply. The bactericidal action of complement on the sensitized microbes also assists in many cases (p. 181). It is this process that brings many acute infections to a rapid end in a crisis of microbial destruction.

But it is by no means all infections that give such a rapid and satisfactory response. In chronic infections like tuberculosis, syphilis, and leprosy the production of specific antibodies is slow and feeble, and there generally ensues a long-drawn struggle between parasite and host, which may settle down into the chronic, fluctuating equilibrium of 'infection-immunity'. Again, the essentially endotoxic organisms (e.g. *Neisseria*, *Haemophilus*) are far weaker stimulants to antibody-production than the exotoxic species such as *C. diphtheriae* or *Cl. tetani*.

The counter defences of bacteria. We have learned in recent years to attribute virulence largely to the chemical composition of the bacterial body, and particularly of its external layers. Most pathogens in their virulent, 'smooth' phase possess a special ectoplasmic or capsular constituent which resists the action of phagocytes, and there is evidence that this is capable of increase under the stimulus of the living tissues (p. 24). Moreover, during the struggle between microbe

and host a continual elimination of the less resistant microbes must tend to raise the average virulence to the maximum of which the species is capable. A very interesting form of defence by changes of antigenic composition has been demonstrated in relapsing fever (p. 186).

Active immunity. Recovery from infection leaves a heightened resistance due to (1) the persistence of circulating antibodies, which may be of short duration or may continue for many years, and (2) an increased specific reactivity of the antibody-producing tissues, enabling them to respond more rapidly and intensively to a subsequent stimulus by the same antigen. This has been shown in the case of diphtheria toxin to persist long after the disappearance of antibodies from the blood. The duration of active immunity varies enormously in different diseases, sometimes lasting only a few weeks, in other cases for a lifetime. It is seldom, if ever, absolute, but may on occasion be overcome by sufficiently large or repeated doses of highly virulent organisms. It may be accompanied by a persistence of the microbe in some organ such as spleen or lymph-glands (latent infection), and its duration is sometimes limited to the period of persistence of the latent infection (infection-immunity).

Local immunity, natural and acquired. Different tissues have various degrees of natural resistance to different bacterial toxins, as may be deduced from the tendency of a given infection to localize in special organs: e.g. Peyer's patches have a relatively low resistance to *Bact. typhosum*, the bronchial mucosa to *H. pertussis*, and the nerve-cells to tetanus-toxin.

A transitory local immunity of an entirely different kind may be acquired by an area of tissue which has been the site of a local infection. Here the resistance of the infected area rises higher than that of the

rest of the tissue, and of the body in general. This has been proved in streptococcal infections of the skin (erysipelas) and is doubtless true in other cases. It appears to depend upon non-specific local superactivity of the reticulo-endothelial system (p. 241). The same state can in fact be produced by the local injection of non-specific solutions of many kinds, e.g. ordinary broth.

Physiological basis of the response. The specific immunity-response to bacteria must be pictured as a special case of a physiological mechanism for ridding the blood and tissues of useless or disturbing foreign proteins. Thus an animal injected with a harmless dose of egg-albumin or foreign blood-corpuscles develops antibodies against them as readily as against virulent bacteria (see Haemolysins, p. 180 and Precipitins, p. 241).

Antigen-antibody reactions

Antigenic specificity and stereochemical structure. Haptens. One of the most interesting immunological discoveries of recent years is that the specific character of antigens depends on the various comparatively simple chemical groups or radicles (Haptens) which form part of the antigenic protein-complex, and even on the spatial arrangement of these groups. We may recall (p. 66) that the type-specificity of the pneumococci is due to different polysaccharides combined with a common protein. Now it has been shown that if a given chemical radicle, such as diazotized atoxyl, is inserted into a number of different proteins (e.g. rabbit serum-protein, horse serum-protein and egg-albumin), an antiserum made against any one of them will react by precipitation with them all; whereas before the chemical operation the proteins showed no antigenic relationship. Conversely, if various radicles

are inserted separately into a single protein, the antiserums against the resulting compounds will be specifically distinct. Thus, when laevo-, dextro-, and meso-tartaric acids are separately attached to horse-serum protein, and antiserums are prepared against each, precipitation tests show that the serums are sharply specific, in spite of the common protein of the antigens. Continued research on these lines is rapidly widening our understanding of the specificity of antigens and antibodies.

The chemical detachment of a hapten from its mother-protein generally leaves it without antigenic power, i.e. without the ability to induce antibody-production; but it will still precipitate at a very high dilution (e.g. 1 in a million of hapten) the antiserum prepared against the complete antigen. To restore its antigenicity a hapten has only to be reattached to a protein, or even in some cases to a gum. It is thought that the spontaneous attachment to an indigenous protein in the body may account for the antigenic behaviour of certain drugs (p. 254).

The multiplicity of antigens. Cross-immunity. A bacterium causes the production of a separate antibody against each of its antigens. If, for example, it has both specific and group components, the infected body will elaborate antibodies against both. The group antibody acts not only on the microbe that caused its production, but on any species possessing the same antigen. A good instance of this is the partial 'cross-immunity' against paratyphoid fever conferred by inoculation with vaccine of *Bact. typhosum*. Here the common fraction of the somatic (O) antigen (p. 88) is responsible for the effective group antibody. In fact, whenever two bacteria or viruses have a common toxic antigen, a certain degree of cross-immunity is to be expected.

The unity of antibodies. It is now generally held that a single antigen gives rise only to one antibody, which will give all types of antigen-antibody reaction, according to the way the antigen is presented to it: precipitation, if the antigen is in solution; complement-fixation, if the appropriate reagents are supplied; agglutination, if the antigen is a surface-constituent of bacteria. Thus, although we may use terms such as Agglutinin, Precipitin, Bacteriolysin, we do not imply that they are necessarily different antibodies.

Bactericidal action. Though this plays some part in the specific defence-reaction, its role is subsidiary to that of the phagocytes. The principles and mechanism of *bacteriolysis* have already been described in connexion with haemolysis, in Chapter XII (p. 181). *Bactericidal action* is merely another aspect of the same phenomenon. The former term is used when the bacteria are both killed and dissolved, the latter when they are only killed. Bacteriolysis only occurs with a limited number of bacterial species, particularly the Gram-negative rods, such as *Vibrio cholerae* and *Bact. typhosum*. Bactericidal action is much more general.

Pfeiffer's reaction. If some anticholera serum is injected into the peritoneal cavity of a guinea-pig together with an otherwise lethal dose of living *Vibrio cholerae*, and if samples of the peritoneal fluid are withdrawn from time to time, the vibrios will at first appear swollen and partly disintegrated (bacteriolysis), and finally they will disappear altogether. The animal will show no sign of ill health. Exactly the same reaction occurs in an immunized guinea-pig injected with the vibrio only. In the former case the anticholera serum provides the amboceptor, and the guinea-pig the complement; in the latter case the guinea-pig provides both. As we have seen (p. 108), this reaction may be used to determine the species of a suspected vibrio,

since it is negative with vibrios other than *Vib. cholerae*.

Agglutination. This reaction, which has been discussed on pp. 27 and 87, doubtless plays a part in the defence process. The clumping of bacteria will enable phagocytes to ingest more at a time. But it should not in itself be regarded as one of the most important effects of the specific antibody, however useful it may be for serological investigations.

Precipitation, which really includes agglutination, is, as we have seen (pp. 30, 238), the simplest manifestation of the fundamental antigen-antibody combination, which underlies the whole process of immunity. In the form of the toxin-antitoxin reaction it is the essential defence-mechanism against toxic infections like diphtheria and tetanus.

In addition to its applications in bacteriology the precipitin-reaction has medico-legal uses in identifying proteins of human or animal origin. Thus even minor blood-stains on weapons or garments can be examined and the human origin of the blood proved or disproved by tests with human and animal antiserums.

The source, nature, and properties of antibodies

Recent researches have traced the production of antibodies with a high degree of probability to the *reticulo-endothelial system*. This consists of large mononuclear macrophages or 'histiocytes', some of which are fixed (sessile) in the endothelium of the liver capillaries, spleen and lymph sinuses, and a few other organs, while others wander freely in the blood and tissues.

Of the chemical nature of antibodies we know next to nothing, except that they are bound up with the globulin fraction of the plasma-proteins. In the horse, the animal generally used for the production of

therapeutic antiserums, the globulin concerned has a large molecule, as shown by ultracentrifugalization; in the rabbit, it is considerably smaller, and is therefore more readily diffusible and probably has a quicker protective action. For this reason rabbits are now being used by some serum-producers in place of horses.

The side-chain theory. Ehrlich pictured the surfaces of the antibody-producing cells as possessing protein complexes with numerous chemical side-chains which, by means of their unsatisfied groups or *receptors*, take part in the normal nutritional process. Toxins and other antigens are supposed to possess a combining affinity for certain of the receptors, and the act of combination, assuming that the cell is not much damaged thereby, stimulates it to produce more receptors of the same type. These, if produced in excess, are thrown off into the blood, and represent the free, circulating antibody (Fig. 31). Since experiment shows that the combining power of toxins is to a large extent independent of their toxic properties (see 'Toxoid', p. 51), a toxin is considered to be composed of two parts, a *haptophore* (grasping) group and a *toxophore* (poisoning) group. The change from toxin to toxoid involves a loss or deterioration of the toxophore group, the haptophore group remaining intact. Further, since haemolysins and bacteriolysins combine at the same time with antigen and complement, they are figured as having two haptophore groups (see Fig. 31).

The combination of antigen and antibody was represented in this theory as a chemical reaction in simple proportions. But as experimental knowledge progressed a number of phenomena were observed which obstinately refused to fit into the theory. One of the most striking of these was recorded by Danysz.

The Danysz phenomenon. If a certain dose of toxin is added *all at once* to a given quantity of antitoxin, it

is exactly neutralized; but if it is divided into two parts and these are added *one after the other* to the antitoxin, with a short time in between, the mixture

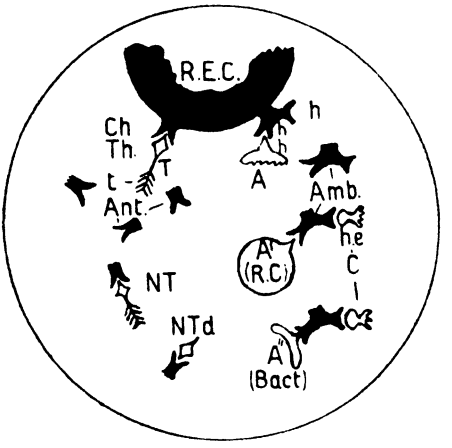

FIG. 31. Ehrlich's theory of Antigens and Antibodies. Diagram of main features

R.E.C. = Antibody-forming (reticulo-endothelial) cell showing two side-chain receptors of different types. h = haptophore groups. Ch. = Cell-haptophore. T = toxin. Th. = Toxin-haptophore. t = toxophore group. Ant. = Antitoxin consisting of free cell-haptophores. NT = Neutralization of toxin by antitoxin. NTd = Neutralization of toxoid by antitoxin. A = Antigen (foreign protein from red cells, bacteria, &c.). A' (R.C.) = Red-cell antigen. A'' (Bact.) = Bacterial antigen. C = Complement. e = ergophore group of complement.

remains toxic, i.e. some of the toxin is not completely neutralized. Now the only satisfactory explanation of this is that when the toxin is added all at once each molecule secures just sufficient antitoxin for neutralization; whereas when it is added in two fractions the first takes up more than the neutralizing dose of

antitoxin, so that there is not enough left to neutralize the second. Clearly, then, the combination cannot be a chemical one in simple proportions, since the quantities combining vary according to the relative concentrations in the mixture at any given moment.

This and other troublesome facts caused Ehrlich to complicate his theory until it lost most of its attraction, and further experimental attempts were made to explain the combination of antigen and antibody as analogous to that of weak acids and bases (Arrhenius and Madsen), or as a purely physical adsorption like that of a dye by organic fibres (Bordet). But the advance of the chemistry of colloids and antigens has brought us back again towards Ehrlich's conception, the basic idea of which, that the combination is essentially chemical, daily receives new corroboration.

The most recent view of the process is that on the surface of an antibody molecule there are a number of specific groups to which the antigen can chemically attach itself; and, conversely, that the antigen has a number of groups by which it can become attached to molecules of antibody.

The reaction begins by the combination of the two molecules into minute primary complexes (Fig. 32 B). As the reaction progresses larger aggregates are built up by the successive adhesion of molecules of each reagent in turn. If one reagent is present in excess, the molecules of the other may rapidly become completely coated with it, and the growth of the aggregates is stopped at an early stage (inhibition or prozone-phenomenon (Fig. 32 A and C).

In their original state of colloidal solution both the molecules are electrically attracted by molecules of water, which keep them in solution; but as the specific combination progresses this bond is loosed, and the aggregate passes from solution into suspension.

Owing to the possession of identical, negative charges they would tend to repel each other and so remain in suspension, were there no ionized salts in the surround-

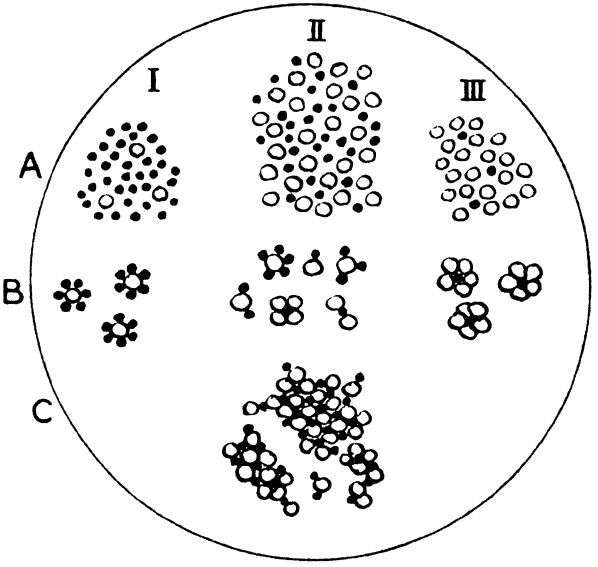

FIG. 32. Combination of Antigen, ●, and Antibody, ○, according to the 'lattice theory'.

A. Mixtures: I, excess of antigen; II, optimal proportions; III, excess of antibody.
B. Formation of primary complexes in the three mixtures.
C. Continued combination, aggregation, and flocculation in II only; inhibition in I and III.

ing fluid; but in the presence of salts the charges are so reduced that the aggregates coalesce into visible floccules and pass completely out of suspension. In the precipitation-reaction the antibody forms aggregates with colloidal antigen; in agglutination, with

whole bacteria. Thus the reaction, whether it take the form of precipitation or agglutination, is generally said to occur in two stages: first, the specific combination, and second, the flocculation by electrolytes. If all electrolytes are removed by dialysis visible flocculation does not occur, though the primary combination has taken place, as can be shown by centrifugal removal of the colloidal aggregates. But the reaction may equally well be regarded as a continuous process in which the specific combination acts in conjunction with the reduction of charge by electrolytes, so that no separate stages are distinguishable. (Marracks 'lattice' theory).

This view of the process clearly admits of a chemical combination in variable proportions according to the concentration of the two reagents, and thus harmonizes and amplifies the earlier theories of Ehrlich, Arrhenius and Madsen, and Bordet.

Dissociation of the antigen-antibody compound can occur if the mixture is diluted or frozen, but the longer the time allowed for combination the less is the tendency to dissociate. Serious misadventures have occurred from the injection of supposedly neutral diphtheria toxin-antitoxin mixtures kept in cold storage.

Avidity of antibodies. Some specimens of antitoxin combine with toxin less rapidly and firmly than others; possibly owing to a deficiency in the number or chemical specificity of the combining groups. Such antitoxins are not suitable for therapeutic use.

Immunity in virus-diseases. The same main agents, antibodies and phagocytes, operate here as in bacterial infections. But in certain cases, such as the neurotropic virus-diseases, it is difficult to prove that they are actually at work and hard to understand how they can operate (p. 219); yet the resulting immunity

is solid. The theory has therefore been advanced that the infected cells after recovery are left with a 'Cellular immunity', independent of antibodies. But if so, we have no inkling of its mechanism.

The main peculiarity of viruses is their intracellular habit, which gives rise both to such differences as exist between bacterial and virus infections, and between the types of immunity they produce. A striking form of 'infection-immunity' has been demonstrated in potato-plants, which, when harbouring an avirulent virus, are resistant to infection with a more virulent race of the same virus. Similarly, monkeys can be made refractory to the yellow fever virus by inoculating them just previously, or even simultaneously, with an attenuated strain. It seems that the cells are 'blocked' by the harmless virus and cannot take up enough of the other to infect them. It is even possible that the immunity to viruses, which is on the whole more solid than bacterial immunity, though with important exceptions (e.g. influenza), may be due to an enduring post-infective symbiosis of virus and cell. Alternatively, it may be that much smaller residues of antibody are needed to protect cells from penetration by a virus than to protect the blood and tissues from bacterial invasion.

Prophylactic and therapeutic inoculations: Vaccines and Antiserums

The materials used in the specific prophylaxis and treatment of infective diseases may be divided into (1) Those that induce *active immunity*, i.e. stimulate the subject to make his own antibodies. (2) Those that confer *passive immunity* by virtue of the antibodies they contain. These procedures are all popularly included in the term 'inoculation'.

Active immunization and therapy by means of vaccines. Suspensions of living or killed bacteria or viruses, prepared for injection into man or animals, are termed vaccines; the name being derived from vaccinia (Lat. *vacca*, a cow), which was the first material used in the prophylaxis of an infective disease. A vaccine made with the patient's own microbe is called *autogenous*.

The *prophylactic* value of vaccines is estimated according to their ability to (*a*) prevent attacks of the disease, and (*b*) reduce the death-rate of those attacked. In a number of diseases, such as small-pox, typhoid fever, rabies, and plague, the efficacy of the procedure has been scientifically proved by the compilation of statistics showing the incidence and fatality of the infection in equivalent groups of inoculated and uninoculated persons.

But with regard to the *vaccine treatment* of already established infections no comparable records are available, and it cannot be said that the procedure has been scientifically proved to have any curative value. This, of course, does not imply that it has in fact no curative value. Most physicians and pathologists think it has, though they differ greatly about its proper scope and limitations.

On the theoretical side it is difficult to see why a person who is already well 'dosed' with a living bacterial antigen should benefit from additional doses of the same substance in the dead state. A common explanation is that in chronic localized (walled-off) infections the antigen does not circulate sufficiently to act as an efficient stimulus to antibody-production, and that the specific vaccine provides the necessary additional stimulus. There is, however, no proof of this; nor is it inherently very probable. We cannot, in fact, be sure that the effects are always specific. There are many

instances on record of striking therapeutic effects following the injection of vaccines made with bacteria unconnected with the disease; or even of non-bacterial proteins. Such substances doubtless act by giving a non-specific stimulus to the cells of the reticulo-endothelial system. Whatever may be the benefits of vaccine-therapy in suitable cases, there can be no doubt of the harm it can do if unwisely applied: e.g. in too large doses, or in the acute stage of infections, when the defence-mechanisms are already stimulated to the utmost.

Passive immunization and serum-therapy. Antiserums are prepared from the blood of horses or other animals subjected to a course of injections with a vaccine or a toxin. *Antitoxin serums* (diphtheria, tetanus, scarlatina, &c.) are produced by immunization with toxin or toxoid. They have been amply proved to confer *antitoxic immunity* but they have little or no action on the bacteria themselves. *Antibacterial serums* are prepared by immunization with vaccines. The *antibacterial immunity* they afford is probably due in the main to their opsonic action, certainly not to antitoxin. The efficacy of such serums has been proved by sound statistical methods in anthrax, plague, certain pneumonias, and some other diseases; but most infections have proved refractory to serum-therapy.

Human 'convalescent' and 'adult' serum. The uses of these in measles and poliomyelitis have already been mentioned (pp. 222, 217). It is important to realize that neither tests for bacterial sterility nor added antiseptics ensure the absence of living viruses.

Oral immunization. By feeding with killed vaccines of certain organisms, e.g. *Bact. dysenteriae* (*Shiga*), animals can be protected against an otherwise lethal dose of the living organism. In man, typhoid vaccine

by the mouth regularly gives rise to the O type of agglutinin in the blood, which is evidence of a general immunity response.

Oral immunization against enteric fever, dysentery and cholera is still on its trial. Up to the present the evidence suggests that some protection is afforded, but probably less than that given by injection into the tissues.

Intranasal instillation of formalin-killed pneumococci in rabbits has recently been shown to produce solid specific immunity, with or without measurable antibodies. This may prove a promising line for the immunization of man.

Hypersensitiveness. Anaphylaxis

In order to produce immunity, the stimulation by an antigen must be of an adequate intensity and duration. A single, slight antigenic stimulus may give rise, not to immunity, but to the intermediate state of *hypersensitiveness*. Any antigen, whether toxic or non-toxic, will serve to produce the phenomenon. If, for example, a guinea-pig (or other animal) is injected subcutaneously with a single small dose of horse-serum, and *after a minimum of ten days* a second and larger dose (in itself harmless) is injected into its blood, the animal is taken ill almost immediately, passes its faeces and urine spasmodically, and dies in a few minutes in convulsive asphyxia. This phenomenon is known as *anaphylactic shock*. It has been shown to be chiefly due to a spasmodic contraction of unstriped muscle; in guinea-pigs, especially in the bronchioles; in rabbits, particularly in the pulmonary vessels. It can, in fact, be prevented, and the life of the animal saved, by the timely injection of adrenalin or atropine. In different animals the manifestations of anaphylaxis differ both in kind and intensity; by a happy chance

man is one of the least susceptible. In the guinea-pig a lowering of the coagulability of the blood and an accumulation of leucocytes in the lung-capillaries are two of the most constant features. Generally speaking the hypersensitive or 'anaphylactic' state lasts for at least one or two years.

The 'partial-immunity' theory of anaphylaxis. The weak primary stimulus causes a relatively feeble production of specific antibody, which reaches a maximum in about 10 days. The small quantity of circulating antibody is continually absorbed and retained by the tissue cells, so that its concentration in the blood remains very low. When a considerable dose of the antigen is now injected directly into the circulation, it causes precipitation on and in the cells, and gives rise to a violent functional disturbance (e.g. contraction of muscle). If the concentration of circulating precipitin had been sufficiently high, as in the fully immune state, the antigen would have been precipitated and neutralized before reaching the sensitized cells, and no shock would have resulted.

Experimental basis of the theory. A *passive hypersensitiveness* can be induced by injecting into a normal animal the serum from an actively hypersensitized animal. The serum contains precipitins, which sensitize the cells of the injected animal to a subsequent injection of antigen.

A further proof that anaphylaxis depends on an antigen-antibody reaction is found in the invariable sudden disappearance of complement from the blood, due to its fixation by the precipitated compound (p. 182).

That the reaction really takes place in the cells and not in the blood is proved by the fact that plain muscle-tissue (e.g. uterus) excised from a hypersensitive animal and perfused with Ringer's fluid contracts

spasmodically on the addition of specific antigen to the fluid (Schultz-Dale reaction).

Desensitization. The hypersensitive or anaphylactic state can be removed by the injection of one or more very small doses of the antigen, insufficient to cause shock. The mechanism of this is not clear, but it seems to be due to a partial neutralization of the antibody in the cells, without dangerous precipitation, which blocks the way against a subsequent acute intracellular reaction.

Histamine shock. The fact that *histamine* when injected into normal animals causes a shock exactly like anaphylaxis, suggests that the latter may be due to the liberation of this substance by the damaged cells. This is supported by the salutary effect of adrenalin and atropine (p. 250), whose pharmacological action is opposite to that of histamine.

Skin hypersensitiveness. Allergy. A small quantity of antigen injected intracutaneously in a hypersensitive subject gives a brisk local inflammatory action, whereas it has no effect on a normal subject. The tuberculin, mallein, and similar tests for specific hypersensitiveness to bacterial antigens are based on this principle. The term *Allergy* is practically a synonym for hypersensitiveness in the widest sense.

An analogous but not identical condition is the *natural hypersensitiveness* of certain individuals to a variety of animal and vegetable proteins, drugs, and other non-protein chemicals. Such an individual when exposed to his special poison, whether it be grass pollen, hair and epithelium of horse or cat, shell-fish, strawberries, quinine, iodine, or a cocaine-derivative, suffers from acute discomfort, urticarial rashes, catarrh (hay-fever) or asthma, and sometimes from a kind of anaphylactic collapse. In idiosyncrasies due to pollen, dust, or food it is often possible to trace the irritating

matter by a series of skin-tests with a range of likely substances, and the hypersensitiveness can sometimes be reduced by a series of specific immunizing injections.

These *idiosyncrasies*, as they are often called, differ from acquired hypersensitiveness in being spontaneous, inborn, and hereditary in the sense that a tendency to idiosyncrasies in general runs in families. In the same category is the *serum-sickness* which often follows injections of horse-antitoxin. It consists of urticarial rashes, swellings of the joints, and other troublesome symptoms; and when the serum is given intravenously an alarming but temporary collapse is occasionally seen. Serum sickness may be accentuated to a dangerous degree in persons who have had previous antitoxin treatment, and there are, in fact, a number of cases on record of what appears to have been true anaphylactic death after intravenous injection in sensitized subjects.

Theories of idiosyncrasy. It is possible to explain many of the phenomena as the result of the precipitation of small amounts of natural antibodies in the cells by the provocative substance, just as anaphylaxis is explained as due to the precipitation of acquired antibody by the specific antigen. Natural hypersensitiveness, like anaphylaxis, can be specifically transferred by injection of the serum of a hypersensitive subject into a normal one, but a difficulty arises in that the serum will seldom, if ever, give precipitation *in vitro* with the provoking substance, as it nearly (but not quite) always does in anaphylaxis.

Though this may simply be due to quantitative differences, it has led to an alternative theory which supposes that reactive bodies other than true antibodies are concerned in the idiosyncrasies. These are termed *reagins*; the provoking substances are called *atopens*, and the state to which they give rise is termed

Atopy. This theory draws support from the apparently non-antigenic character of some of the 'Atopens', viz. drugs and other chemical substances. It is, however, reasonable to believe that these may act like haptens, which will give an intracellular precipitate with traces of any natural antibody capable of combining with them. They can, in fact, induce specific hypersensitiveness in apparently normal subjects, a phenomenon which can be explained by their acquisition of antigenic character on coupling with a serum protein (p. 239). Thus it is at present not clear that the postulation of special 'reagins' and 'atopens' is necessary for an explanation of the idiosyncrasies.